A PROSPECTIVE CASE STUDY OF ESTROGEN/PROGESTERONE/ HUMAN EPIDERMAL GROWTH FACTOR 2 RECEPTOR STATUS IN BREAST CARCINOMAS

This dissertation is submitted to PSG Institute of Medical Sciences and Research in partial fulfilment of the regulations for the M.S (General Surgery) Degree Examination, April 2015

By

Dr. SHREE VISHNU SIDDARTH.R

Done under the guidance of

Dr.Balashanmugam.T.S
Professor of Surgery
PSG IMS&R
Coimbatore – 641004

CERTIFICATE

This is to certify that this dissertation entitled **"A PROSPECTIVE STUDYOF ESTROGEN/PROGESTERONE /HUMAN EPIDERMAL GROWTH FACTOR 2 RECEPTOR STATUS IN BREAST CARCINOMAS"** is a record of bonafide research work done by Dr.Shree Vishnu Siddarth.R, under my guidance and supervision in the Department of General Surgery, PSG Institute of Medical Sciences and Research, Coimbatore – 641004.

Dr.Balashanmugam.T.S,
Professor of Surgery,
PSG IMS&R,
Coimbatore – 641004.

Dr.S.Ramalingam,
Principal,
PSG IMS&R,
Coimbatore – 641004.

DECLARATION

I, Dr. Shree Vishnu Siddarth.R, solemnly declare that this dissertation **"A PROSPECTIVE STUDYOF ESTROGEN/ PROGESTERONE/HUMAN EPIDERMAL GROWTH FACTOR 2 RECEPTOR STATUS IN BREAST CARCINOMAS"** is a bonafide record of work done by me in the Department of General Surgery, PSG institute of Medical Sciences & Research, Coimbatore, under the guidance of Dr.Balashanmugam.T.S, Professor of Surgery.

This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfilment of the University regulations for the award of MS Degree (General Surgery) Branch-I, Examination to be held in April 2015.

Place: Coimbatore

Date: 30.09.2014 (Dr. Shree Vishnu Siddarth.R)

ACKNOWLEDGEMENT

I would like to express my gratitude to the Institute and to our Principal for permitting me to conduct this study.

I would like to sincerely thank Dr.Balashanmugam.T.S, Professor of surgery for his guidance, motivation and supervision. His mentorship was of paramount value all through the study.

I would also like to thank the faculties of the surgery department for the persistent guidance and supervision.

My utmost respect and gratefulness to all my patients who were cooperative and helpful in providing data for my study.

My thanks are also to my colleagues, Interns and Staff Nurses for the considerable help extended to me.

Finally and most importantly, I would like to thank my family for their support, encouragement and unwavering love which has been the pillar of my strength.

CONTENTS

INTRODUCTION

INTRODUCTION

Breast carcinoma is the second most common type of malignancy diagnosed in female's next onlyto cervical carcinomaworldwide. In majority of the developedcountriesit is the most frequently encountered malignancy and one of the major causes for cancer related deaths[1]. Breast carcinoma has been extensively studied upon in the modern medicine era and with the availability of vast evidence based data and literature various treatment modalities have been introduced to treat this life threatening disease.

Majority of the breast carcinomas are usually asymptomatic and the usual mode of presentation is an incidental palpable lump or pain and rarely, they present with nipple discharge and skin changes[2]. Breast carcinomas have varying levels of invasion and aggressiveness irrespective of the duration and the metastatic symptom could be the presenting complaint in a few. Even though extensive screening programs and clinical tests are available for early detection of this disease the turnover and outcome still remains poor in developing countries. There is a lack of self-awareness of this life threatening malignancy in our country.

The general approach in case of breast carcinoma is by triple assessment test combining the clinical finding with that of the radiological and pathological correlation remains as a standard guideline for breast carcinoma. The categorisation is based upon the triple assessment test[3]. Liver function assessment, X-Ray, ultrasonography and bone scan aid in staging the disease.

Histo-pathological correlation along with immunohistochemistry analysis has introduced another modality of treatment with hormones. The usual routine of management in breast carcinoma is with surgery, radiotherapy and chemotherapy depending on the stage of the disease[4]. Though each modality of treatment has its own pros and cons, combined approach is the therapy of choice leading to less toxic therapy with a good outcome and better prognosis.

Breast carcinoma management is reaching new heights with its vast research interest and data availability. Numerous centres throughout the world are promoting screening programmes and self-examination test to detect the disease as early as possible

In this study we have concluded our experience and interest with the breast carcinomas and its immunohistochemistry analysis in different stages of disease process and we aid to improve the current knowledge with our encounter with this life threatening disease.

AIM OF THE STUDY

AIM OF THE STUDY

- To analyze the Estrogen/ Progesterone/ Human epidermal growth factor 2 receptor status in breast carcinomas and comparing them with that of the staging

REVIEW OF LITERATURE

REVIEW OF LITERATURE

History of Breast carcinoma:

The first documented historical note about breast carcinoma is by the Egyptians in 1600 BC which is titled as 'CASE 45- INSTRUCTIONS CONCERNING TUMOURS ON HIS BREAST' which elaborates that the tumour is bulging and cold and this ailment has no treatment[4,5].

Aurelius celsus in 30 AD wrote 'De Medicina' which contains the description of a breast cancer.He described it as an irregularly fixed swelling with dilated veins and ulceration, he also categorised them into 4 varieties. The greek are credited for their first ever surgical approach to breast carcinoma by applying heat to the tumour and escharing[5] it.In the renaissance period the amputation of the breast was considered to be the treatment of choice for the breast carcinoma and they even suggested that the axillary lump is most likely a sequel of breast tumour.

In the 18th century Henri francois le dran from france suggested that in early stages breast carcinoma is a localised disease process and later it spreads to the lymphatics[5].In 1829 the term 'metastasis' was introduced by Dr.Joseph reclaimer. In 1874 Paget published a paper on the 'disease of the mammary areola a preceding cancer of the mammary gland' which described the involvement of nipple with eczematous

changes along with malignant lactiferous ducts (Paget's disease of the nipple)

\ Undoubtedly the 20th century can be called the revolutionary period in the history of breast carcinoma with its pioneers and modern medical and surgical practice[6]. William Halsted proposed that the specimen should be removed in one piece as he suggested that the manipulation would increase the risk of spread and introduced the Radical mastectomy which was practised without argument for more than 70 year. Later, Patey and Dyson introduced the approach of modified radical mastectomy. Dr. Donald .L Morton introduced the concept of sentinel lymph node biopsy revolutionizing the indication and staging work up of breast carcinomas[6].

The introduction of radiotherapy as a modality of treatment of breast carcinomas was popularised by Dr.William Stone and he claimed that radiotherapy is superior to surgery in case of advanced malignancy[6]. Bernard Fischer through his dedicated work on breast cancer explained the concept of systemic disease of this tumour and suggested that breast carcinoma is a systemic disease and requires multimodality approach[7].

The hormonal receptors namely the estrogen and progesterone were demonstrated in 1970's and since 1980's tamoxifen has been used as to treat breast carcinomas. In late 20th century, several studies revealed

an equal benefit with both neo adjuvant as well as adjuvant chemotherapy regimens.

The mammogram patterns of all breast carcinoma patients were studied for a decade at the Philadelphia institute and their accuracy in diagnosis had been published[7]. Later, in 1962 Dr.Egan introduced the views of imaging and since 1970's mammogram is considered as an important tool in diagnosis of breast carcinoma[7].

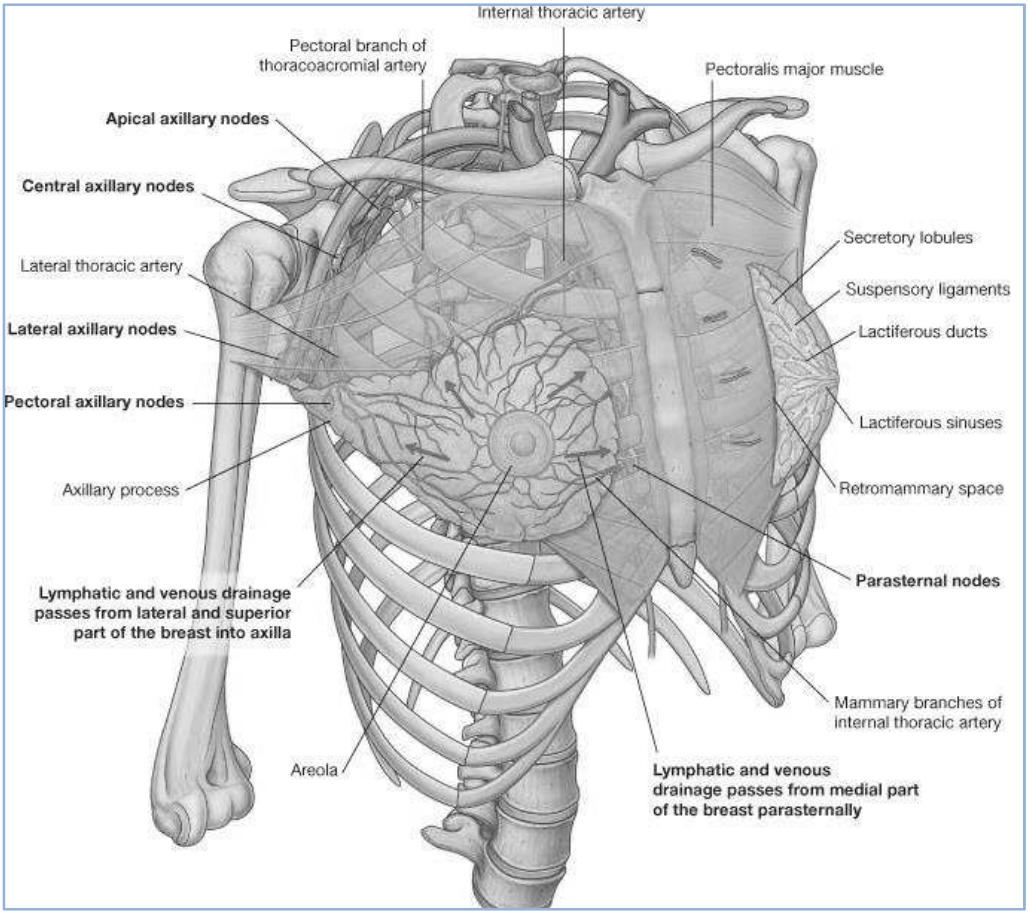
Date	Reconstructive procedure
1896	Latissimus dorsi flap
1900	Skin grafts
1958	Harvested skin grafts with motorised brown dermatome
1960s	Tubed skin flaps
1964	Silicone breast implants
1970s	Myocutaneous flaps
1982	TRAM flap

The breast reconstruction procedures, have been modified concurrently with advent of new techniques and implants and has a good outcome for a postsurgical patients psychologically as well as aesthetically[8].

TNM staging was proposed in 1944 and is on standard practise since then. In the late 20th century the identification of BRCA1 and BRCA 2 is promising and genetic based therapies are awaited to treat this disease[9].

Anatomy of the Breast:

The breast tissue is embryologically derived and matures as a modified sweat gland. They are located within the 'milk line' which extends from the axilla upto the inguinal region. During the fertile period owing to the ovarian hormone synthesis structural changes occur and post menopausally[11], they involute and diminish in structural volume, contour and form due to changes in hormonal levels.



ANATOMICAL LOCATION AND EXTENT OF THE BREAST

A mature breast extends from the second to seventh rib vertically and horizontally from the sternal border upto the anterior or mid axillary line[11].The 'axillary tail of spence' is a continuation of breast in to the axilla. The upper outer quadrant is voluminous compared to other quadrants. They rest upon the pectoralis major, serratus anterior and external oblique muscle[12].

Each breast consists of 15 to 20 tubuloalveolar glandular structures supported by fibrous connective tissue and adipose tissue.They do not possess a capsule but the gravitational and longitudinally support is provided by the extension of connective tissue inbetween the lobes and lobules.

A retromammary bursa is formed by the fusion of the fascia of the breast with that of the pectoralis muscle which makes it mobile over the chest wall. The coopers ligament is a thickened fibrous connective tissue travelling from the dermis to the deep layer of the superficial fascia and is responsible for the contour of the breast[11].

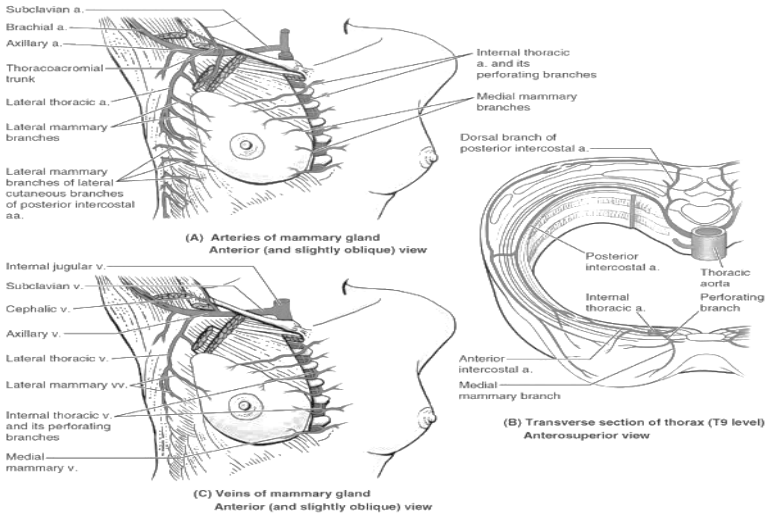
The axilla consists of a pyramidal compartment situated between the thoracic cavity and the upper limb. It communicates with the posterior triangle through the cervicoaxillary canal[12]. The anterior wall is formed by the pectoralis major and minor muscle, posteriorly by the subscapularis, teres major and latissimus dorsi. Laterally by the bicipetal

groove and medially by the serratus anterior. The pectoralis major is covered by the pectoral fascia whereas the pectoralis minor and subclavius is covered by the clavipectoral fascia or costocoracoid fascia extending from the axilla to the clavicle.Thepectoralis minor divides the axillary artery into three segments as per their location – medial ,posterior and lateral to the pectoralis minor muscle[11,12].The axillary artery is in close continuity with the brachial plexus and the divisions are named as per their relation to the artery.

There are three nerves which are of surgical importance namely the long thoracic nerve which supplies the serratus anterior and runs along the medial border of the axilla through the cervicoaxillary canal. Accidental injury to it causes winging of scapula. The other nerve is the thoracodorsal nerve which originates from posterior cord of brachial plexus and supplies the latissimus dorsi muscle and accidental division causes motor dysfunction and results in atrophy and further manipulation for myocutaneous flaps are not feasible[12]. The intercostobrachial nerve provides sensation to the apex and lateral axilla.

The main blood supply to the breast is by the internal mammary artery, posterior intercostal artery and branches of the axillary artery[11]. The thoracodorsal branch from the subscapular artery is of surgical importance as it may cause significant bleeding during axillary dissection

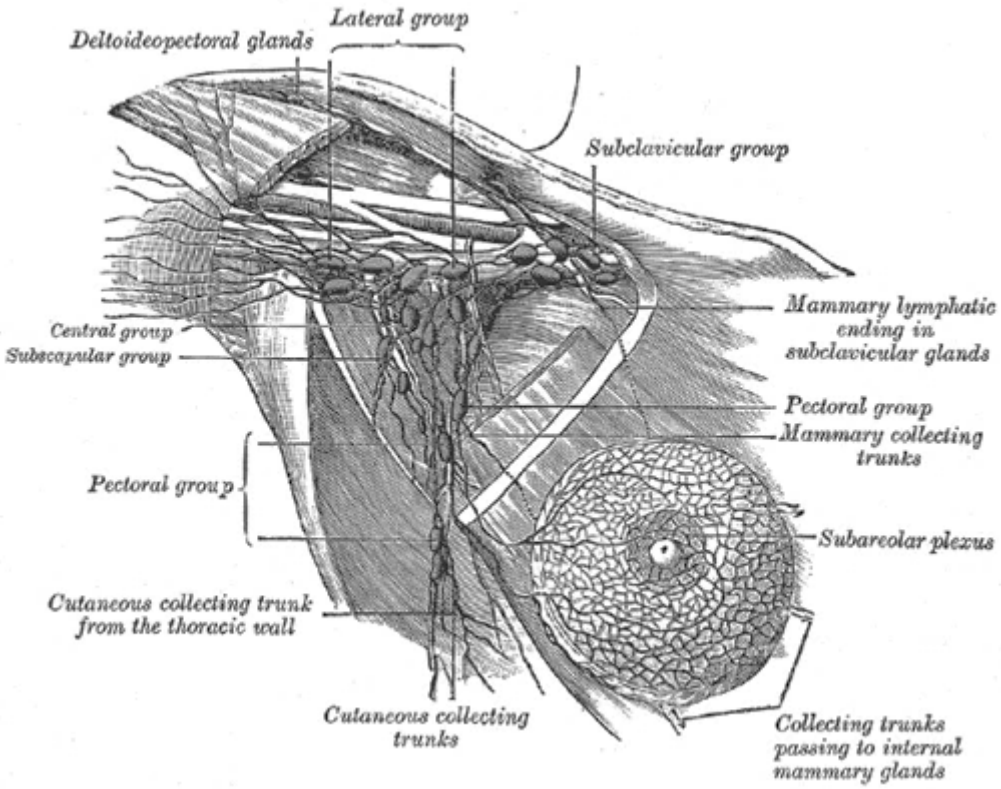
when severed. The venous drainage of the breast is mainly by the internal mammary vein, tributaries of the axillary vein and the posterior intercostal vein. The posterior intercostal vein is in close proximity with the batson's venous plexus and accounts for the vertebral, skull and brain metastases[12].The breast has its innervation from the intercostal and intercostobrachial nerves and its branches which include the lateral and medial mammary branches.



PICTURE SHOWING THE ARTERIAL AND VENOUS SUPPLY OF THE BREAST

The lymphatic drainage of the breast is predominantly through the axillary group of lymph nodes, which consists of six groups of lymph node at three anastamotic sites[11,12]. They include the lateral, anterior, posterior, central, apical and interpectoral group of lymph nodes. The lymph nodes are categorised as per their relationship with the pectoralis minor as level I, II and III which lies lateral, behind and medial to it respectively[13].Dr.Sampson Hadley was credited for his recognition of

internal mammary lymph nodes and his theory of metastatic spread through them to the thoracic duct.



LYMPHATIC DRAINAGE OF THE BREAST

Surgical level	Anatomical correspondence
I	Pectoral axillary lymph nodes (anterior)
	Subscapular axillary lymph nodes (posterior)
	Humeral axillary lymph nodes (lateral)
II	Central axillary lymph nodes
	Some apical axillary lymph nodes
	Interpectoral lymph nodes (Rotter's) *
III	Apical axillary lymph nodes

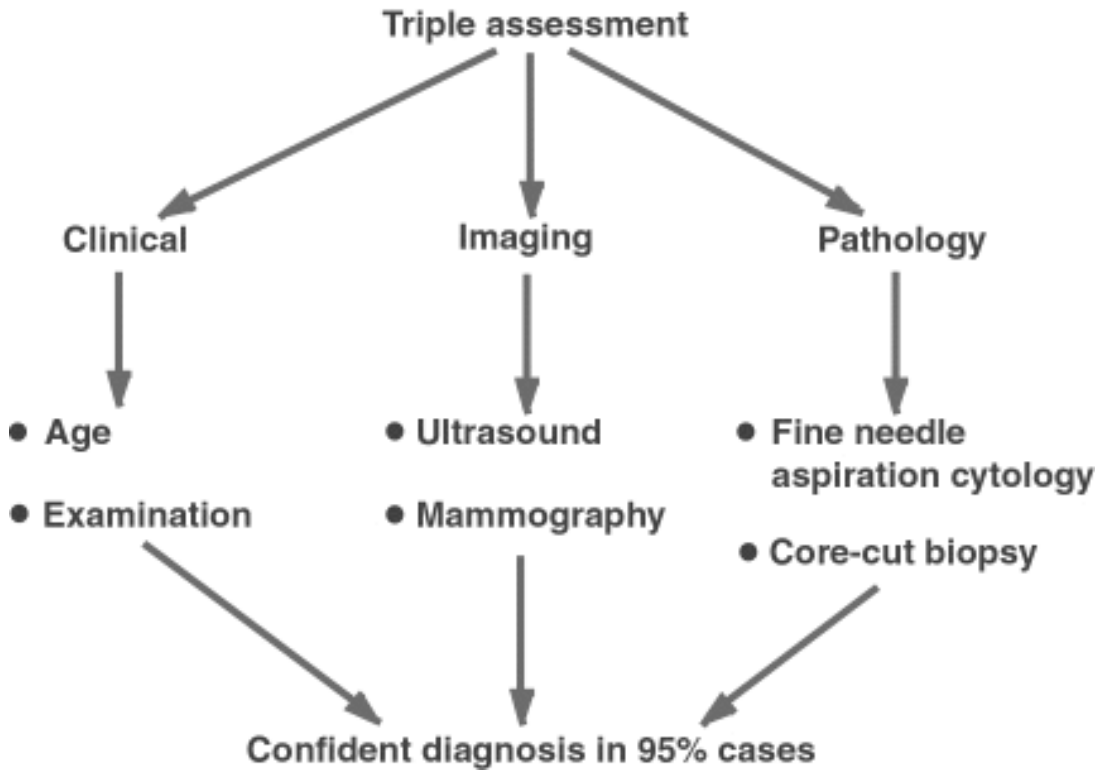
Almost 75% of lymphatic drainage of the breast is through the axillary lymph node and the rest 25% by the internal mammary,

intercostal and the diaphragmatic lymph node[13]. Surgically this drainage pattern is significant as it forms the pillar for the development of sentinel lymph node biopsy technique. However there is no proven exact pathway of lymphatic drainage and chances to miss a positive lymph node is highly possible. This is considered to be one of the biggest drawbacks of sentinel lymph node biopsy[13].

Diagnostic approach in breast carcinomas:

The gold standard diagnostic approach in breast carcinoma till date[14] is the triple assessment test which includes

- History and examination
- Imaging
- Tissue/FNAC



History and physical examination:

The key points in the history should be stressed upon in detail with a patient presenting with breast pathology. They usually present with a palpable lump / pain / discharge / nipple retraction, however 60% of the patient with breast carcinoma are asymptomatic at early stages[14]. Past history of any gynaecological malignancies, previous breast surgeries and irradiation should be enquired in detail. Family history of breast carcinomas increases the risk of developing breast malignancy by fivefold[15]. There are several proven risk factorsfor development of breast carcinoma and all of them are to elaborately taken into consideration in case of a suspected breast cancer. They include the following.

- Female gender
- Increasing age
- Family and personal history of breast cancer
- Previous breast biopsy
    - Proliferative breast disease without atypia
    - Atypical hyperplasia
    - Lobular carcinoma in situ
- Previous thoracic irradiation
- Endocrine risk factors
    - early menarche

    - Late menopause
    - Late or nulliparity
    - Long term hormonal therapy
- Lifestyle factors
    - Alcohol
    - Obesity
- Genetic factors
    - BRCA 1 & 2
    - Ataxia telangiectasia
    - Li-fraumeni syndrome
    - Cowden syndrome

In physical examination the size and location of tumour, extent, fixity, skin involvement helps in concluding the 'T' stage of the disease. Careful examination of all groups of lymph node is mandatory in breast carcinoma and the opposite breast and nipple examination is of utmost importance. The single most important prognostic factor in breast carcinoma is considered to be the lymph node status and the 'N' stage of the disease is ascertained based on this examination. Systemic examination is warranted as breast malignancy is considered to be systemic disease and metastasis should be ruled out by both clinical

examination as well as investigations to ascertain the ‘M’ stage of the disease and to decide upon the modality of management.

The breast malignancy is universally classified by the AJCC, by a combination of the Tumour size, nodal status and metastatic disease into TNM staging. The TNM staging is the same for clinical as well as the pathological except for sub categorising them according to the pathological findings and are denoted by subsets. Depending on the TNM staging the breast malignancy is further classified into four different stages using a combination probability of the same. For management and prognostic purposes the staging is further divided into early breast carcinoma, loco-regionally advanced breast cancer and metastatic carcinoma. They include the following

TNM STAGING[15]

Primary tumour (T)

TX - Tumour cannot be assessed

T0 - No evidence of primary tumour

Tis - Carcinoma in situ

T1 - Tumour less than 2 cm in greater dimension

T1mic - micro invasion 0.1cm or less

T1a - more than 0.1 but less than 0.5cm

T1b - more than 0.5cm but less than 1cm

T1c - more than 1cm but less than 2cm

T2 - Tumour more than 2 cm but less than 5 cm in greater dimension

T3 - Tumour more than 5 cm in greater dimension

T4 - Tumour of any size with direct extension into skin or chest wall

T4a - extension to chest wall

T4b - edema(peau'd orange) or ulceration or satellite nodules

T4c - Both T4a and T4b

T4d - Inflammatory carcinoma

Regional lymph nodes (N)

NX - regional lymph nodes cannot be assessed

N0 - No regional lymph node metastasis

N1 - Metastasis to ipsilateral mobile lymph node

N2 - Ipsilateral fixed lymph nodes

N3 - Metastasis to ipsilateral internal mammary lymph node

Distant metastasis

MX - Distant metastasis cannot be assessed

M0 - No distant metastasis

M1 - Distant metastasis (ipsilateral supraclavicular lymph node is included)

TNM Staging

Modified AJCC cancer staging of breast carcinoma

Stage 0	Tis	N0	M0
Stage I	T1a	N0	M0
Stage II A	T0 T1a T2	N1 N1 N0	M0 M0 M0
Stage II B	T2 T3	N1 N0	M0 M0
Stage III A	T0 T1a T2 T3 T3	N2 N2 N2 N1 N2	M0 M0 M0 M0 M0

Stage III B	T4 T4 T4	N0 N1 N2	M0 M0 M0
Stage III C	Any T	N3	M0
Stage IV	Any T	Any N	M1

- Early breast carcinoma - Stage I and II A&B
- Locally advanced breast carcinoma - Stage III A,B&C
- Metastatic breast carcinoma - Stage IV

Imaging

Over the past century there had been a vast revolutionary change in the field of radiology implementing new techniques and aiding accurate diagnosis in breast carcinomas. The imaging modalities in breast carcinoma include

- Mammogram
- Ultrasound
- Ductography
- CT/MRI
- PET CT/Bone scan

Mammography

In asymptomatic patient screening mammogram is used to detect breast lesions if they are not clinically conclusive or in a patient with nipple retraction or nipple discharge without any palpable pathology or in a patient who had undergone breast conservative surgery for previous malignancy.

Mammogram consists of two basic views the craniocaudal view and the medio-lateral-oblique view. They are checked for asymmetry,calcifications, masses or architectural distortions, they either present individually or in a combination of the above[16]. In case of margins they are described as per their shape (round, oval, lobular, irregular) and margins(circumscribed, microlobulated, obscured, ill defined, spiculated)[16]. Calcifications alone do not suggest the lesion to be malignant as they coexist in normal and benign conditions of the breast.

The American college of radiology has developed the Breast imaging reporting and data system (BIRADS) for classifying breast lesions which is as follows[16]

0	Needs additional image evaluation
1	Negative
2	Benign finding
3	Probably benign finding
4	Suspicious of malignancy
5	Highly suggestive of carcinoma
6	Known carcinoma

Ultrasound

A 7MHz linear transducer is the minimum frequency to image breast lesion. Though mammogram is not the ideal choice of investigation for breast carcinoma it is an excellent adjunct in evaluation of breast cancer when combined with mammogram and few interventional procedures. The accuracy of ultrasound is 100% in breast cysts[17]. The malignant features of lesion in ultrasonography include irregular margins, hypoechoic, posterior acoustic shadowing and vertical growth appearance[17].

Ductography

Nipple discharge and retraction can be evaluated with ductography in which a contrast is injected in to the duct to identify the filling defect[16,6]. But this modality of investigation is not very specific nor sensitive for malignancy

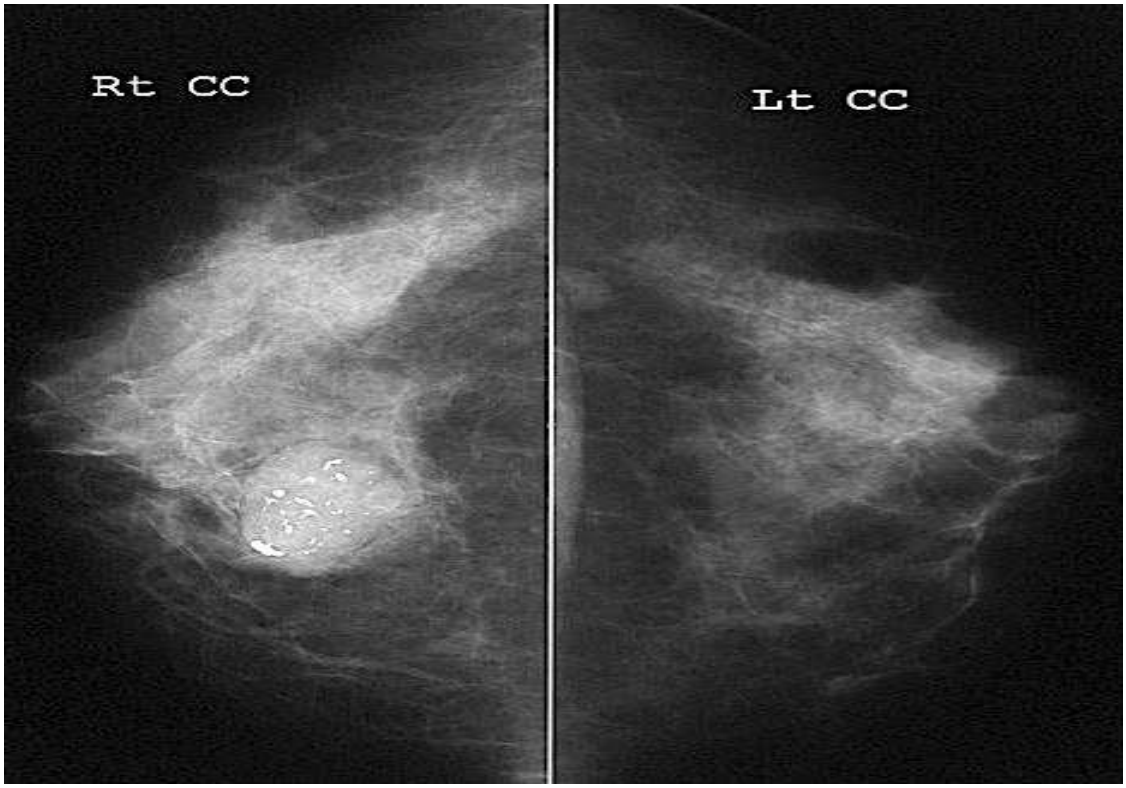
MRI

Though mammogram is the considered as the 'gold standard' for breast imaging, MRI is slowly gaining importance in breast malignancy as it penetrates and density and micromatic changes can be picked up by the MRI.1.5 tesla magnet or greater is to be used in imaging and

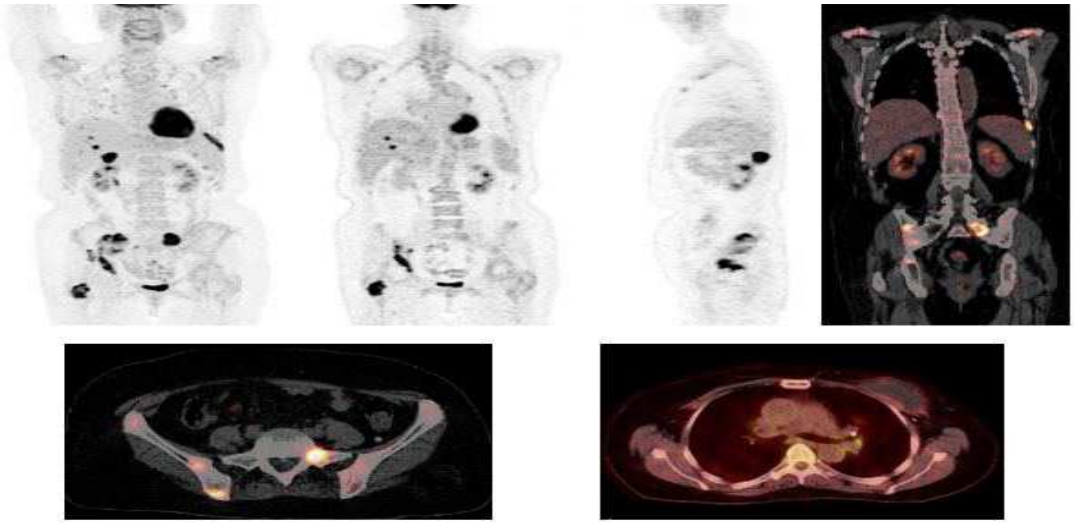
gadolinium is the contrast of choice[18]. The MRI shows post contrast enhancement of malignant lesion

PET CT

PET CT is used in metastatic work up of breast carcinoma. It traces the uptake of FDG polymer by the tumour cells[18]. The limitation of this imaging test is the cost and availability in very few centres in our country.



MAMMOGRAPHIC IMAGING SHOWING A MASS LESION IN RIGHT BREAST WITH MICROCALCIFICATION



PETCT IMAGES SHOWING DISTANT METASTASES

Diagnostic Biopsy

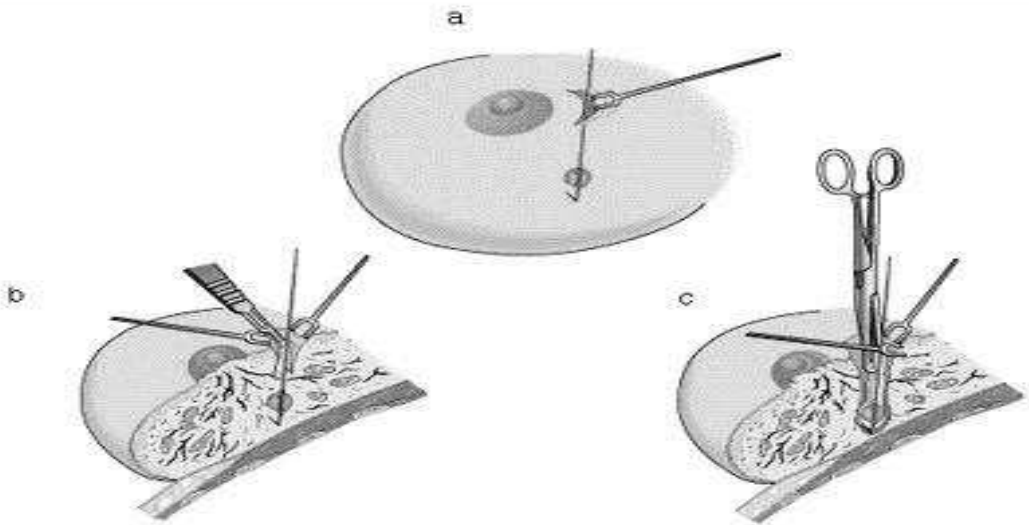
The final step and the most important step in triple assessment is the tissue diagnosis. The tissue diagnosis can be extracted through various techniques. Biopsy is considered to be superior to FNAC, as it can be run for receptor status too[19]. In case of impalpable lesion and accidentally detected tumours, imaging guided biopsy or FNAC is the choice of tissue sampling.

FNAC has got a diagnostic accuracy 80% in breast carcinomas. In the hands of an experienced pathologist the false positive results are almost nil[19]. When false negative results are suspected and when the lump is clinically as well as radiologically suspicious, core biopsy or surgical excision is warranted.

Core biopsy is the evaluation of choice in case of a solid mass and is performed either by palpation or by image guidance. In Stereotactic core biopsy the patient lies prone with breast suspended and compressed, the image is at an angle +/- 15 degree perpendicular to the and the coordinates are accurately marked. The biopsy gun is introduced through these marked sites and several biopsies are obtained and are imaged for microcalcifications[18,19], a clip is placed at the biopsied site so post procedure exact region of specimen obtained can be demarcated.

Ultrasound guided biopsy is easier and simpler than stereotactic biopsy as in shows a live demonstration of the biopsy to the radiologist or surgeon[16,19]. MRI guided biopsies are done for lesions which are only visible on MRI and needs vacuum assistance.

In case of non-palpable lesions pre-operative localisation of the tumour is necessary and it aids the surgeon in complete excision and localisation during the intra operative period. The location of the lesion in mammography is varying as they are subjected to compression. A self retaining hooked wire is placed preoperatively either through mammogram and ultrasonography[19]. The site of location is traced intra-operatively and excised completely with 1cm margin clearance and oriented to the pathologist.



HOOK TECHNIQUE FOR LOCALISATION

Classification of breast carcinoma

Breast carcinomas usually occupy the upper outer quadrant followed by central compartment and then equally by the lower quadrants. In 90% of the cases the carcinoma arises from the ductal epithelium and remaining from the lobular epithelium[20]. The classification is as follows.

1. Non invasive (in situ) carcinoma
    - Intraductal carcinoma
    - Lobular carcinoma in situ
2. Invasive carcinoma
    - Infiltrating(invasive) ductal carcinoma
    - Infiltrating(invasive) lobular carcinoma
    - Medullary carcinoma
    - Colloid carcinoma
    - Papillary carcinoma
    - Tubular carcinoma
    - Cribriform carcinoma
    - Secretory(juvenile) carcinoma
    - Inflammatory carcinoma
    - Carcinoma with metaplasia
3. Paget's disease of the nipple

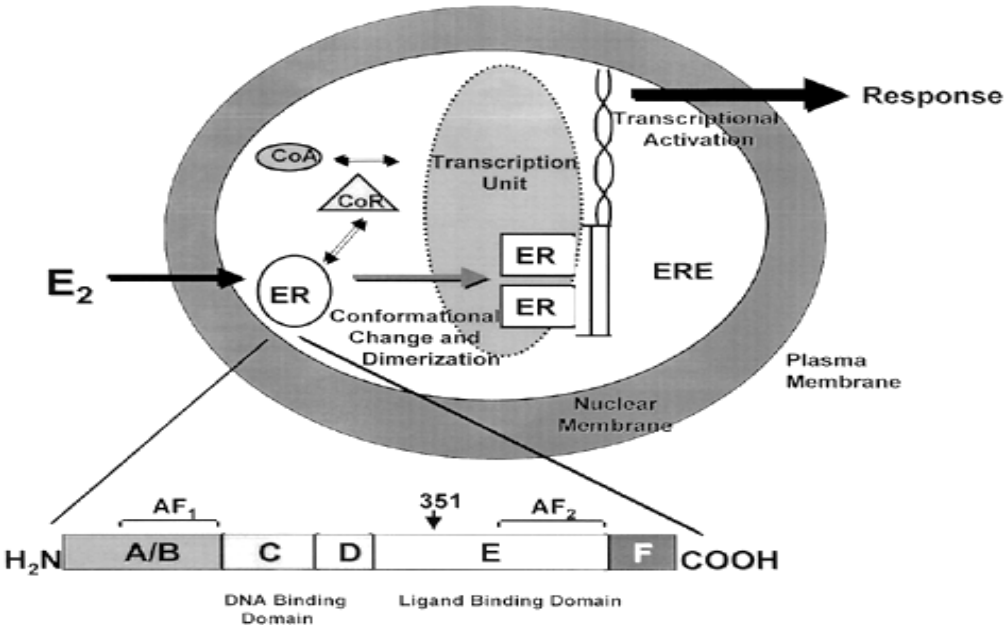
ULCERATIVE GROWTH OF LEFT BREAST

FUNGATING GROWTH WITH METASTATIC DISEASE ON TABLE PICTURE OF TOILET MASTECTOMY

Estrogen and progesterone receptors

In 1960 a radiolabelled estrogen concentrations were studied and was observed that in breast carcinomas concentration percentage was significant and this led to the concept of estrogen and progesterone receptors[21]. ER α and β, PR A and B are primary estrogen and progesterone receptors.

The estrogen and progesterone receptors have their effect in the nucleus by binding with hormones. In a normal breast both ER and PR exhibit positive nuclear immunostaining[21,22], but heterogeneous in nature and tends to vary with each menstrual cycle. In comparison with ER patients who are PR positive tend to have longer duration of disease free interval[22].



ESTROGEN RECEPTOR MODEL

The immunohistochemistry assay for hormonal receptors has become the gold standard in determining the ER and PR levels and has replaced the classical ligand binding assay which is also known as the dextran coated charcoal assay. In recent years with the development of quantitative reverse transcriptase polymerised chain reaction (qRTPCR)[23] ,studies have proven that mRNA assay of ER and PR levels is a better method of determination.

The advantages of IHC assay over dextran coated charcoal assay include the following

- Histological documentation is available
- Tumour nuclei cell heterogeneity can be made
- Rapid turnaround time
- Cost effective
- Small size of sample is adequate
- Can be reported directly visualised or by semi-quantitative methods

The fundamental requirements for hormonal receptor assay by IHC method include the following

- Formalin fixation of breast tissue with a time period of 8 – 72 hours
- 10% neutral phosphate buffered formalin is to be used
- In-vitro diagnostic kits should be used which utilises the following 6F11,1D5 or SP1
- Controls should be used on each run
- Semi quantified results should be based on the percentage of cells staining and their intensity of staining

Recent studies have shown that in pre-analytic assay study minimum formalin exposure time for ER assessment is 8 hours[23]. With overexposed tissue antigen fixation is better but gradually decreases over a period of time. Meanwhile under-fixed tissue study is not worth the assay study.

Conventional processor method is the choice of method for processing the tissue. Though alternate processor methods are available and widely used in many centres, the samples should be validated with that of the conventional processor methods. It's mandatory to document the formalin exposure time and type of formalin used[24]. The antibodies

used in common practise include 1D5, 6F11 and SP1 and are usually targeted against ERα.

Initially it was considered that even 1% of positive staining was reported as positive and adds the beneficial effect of tamoxifen therapy to patient. Several case control studies have proven the beneficiary effect of tamoxifen in high positive ER tumours have an overall survival rate is better compared to the placebo control group[25].

The Allred score quantifies the ER content in the tissue and studies have proven that there is a linear correlation between ER content and the allred score, more significant than reverse transcriptase PCR study. The tumour cells are reported as 0,1+,2+,3+ based on the intensity levels and are multiplied by and ordinal value and given the scoring system as :

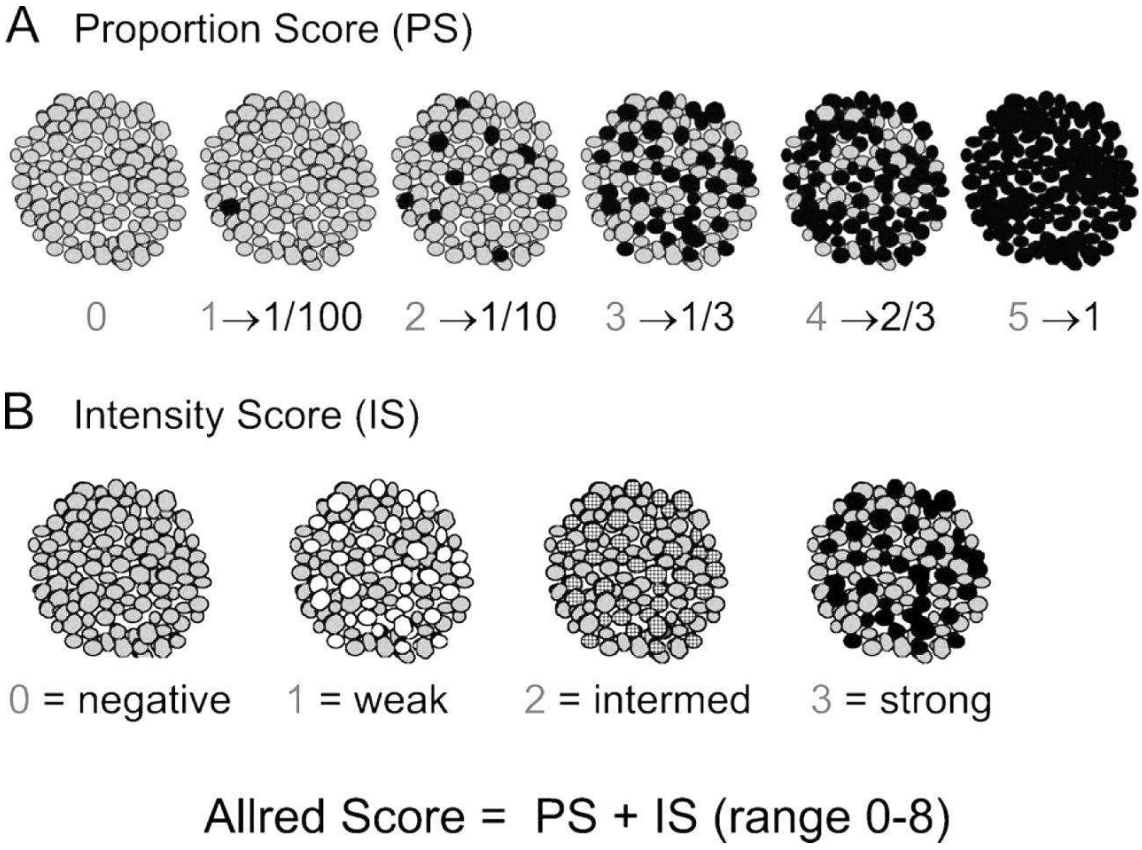
0 = none, 1= weak, 2 = moderate, 3 = strong

The scoring ranges from 0 (no staining) – 300 (diffuse staining)

Allred score = proportion score + intensity score

The progesterone receptors are stained positive for PgR636 and PgR1294 compared to other receptors. The antibodies used in PR staining include the 1A6 and rabbit monoclonal antibody IE2. The ER and PR show parallel correlation and they hardly contradict when used with the same antibody. The IHC staining method in Progesterone receptors is

more heterogeneous than estrogen receptors and show false negative results[23,24]. If there is any doubt or discrepancy in the results with core specimen, assay should be repeated with resection specimen



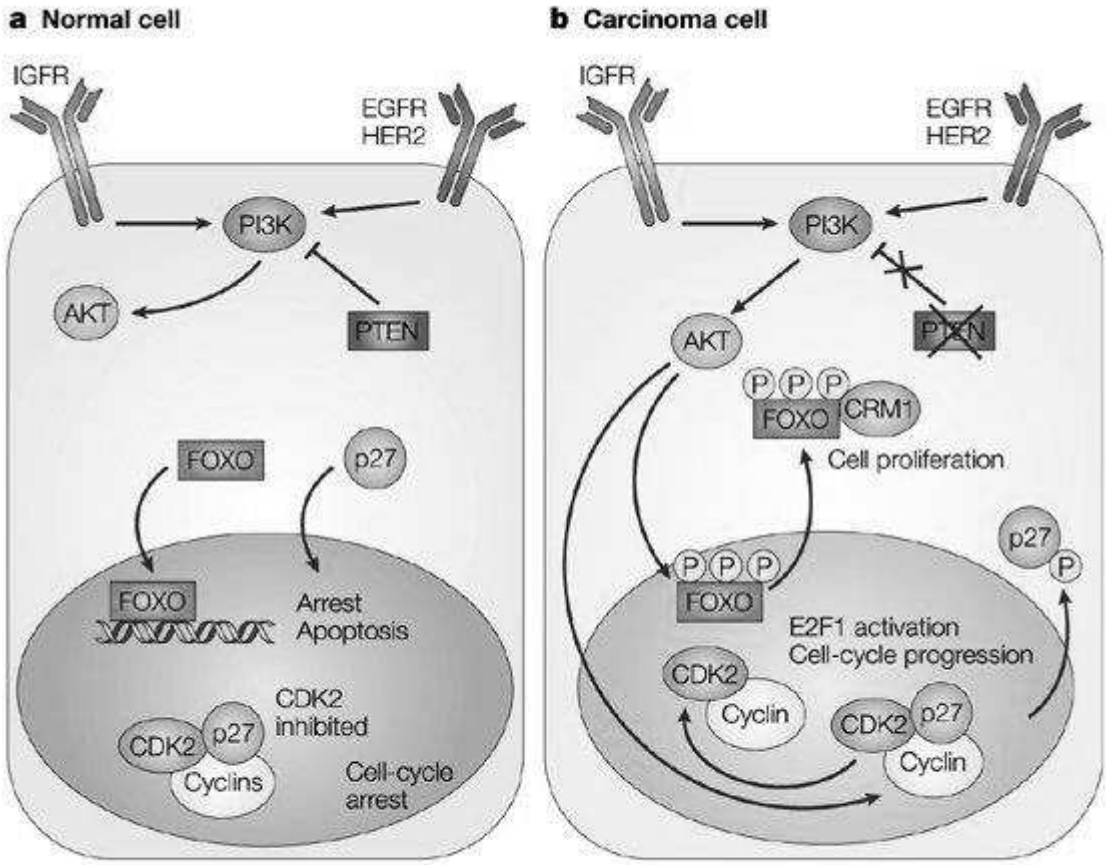
ESTROGEN AND PROGESTERONE STATISTICAL DATA:

- In general with data available from the south east Asian population the expression of estrogen receptor is around 50%[42] .
- The progesterone receptor positivity status is around 35% - 45%[42,43] .
- The estrogen and progesterone positivity usually coexist together irrespective of the histological grading of the disease[43]
- As the age advances the estrogen receptor positivity tends to increase.
- The progesterone receptor positivity breast carcinomas usually tend to be larger in size compared to the other receptor positive statuses[44].
- As per CAP guidelines all receptors which show moderate positivity in allred score are considered positive universally[44]
- When the ER positivity increases the HER2 positivity decreases
- The commonest pattern of receptor status observed worldwide is ER+/ PR+/ HER2-[42,44,47].
- The study on recurrent metastatic breast carcinomas have revealed that there is conversion of receptor status[51]

- If the patient is ER and PR receptor positive the disease free interval is better compared to ER+ and PR – ve tumors[44]
- The IHC assay of hormonal receptors is considered superior to the classic ligand based assay[26]
- In locally advanced breast carcinomas the progesterone positivity tends to be higher than the other receptors
- ER and PR positivity is observed more in postmenopausal women than the other age group
- Obesity tends to increase estrogen positivity[45]
- Familial breast carcinomas are more in favour for ER+ and PR+ status

HER2/neu

The ERBB2 gene is called NEU as it was initially derived from rodent glioblastoma cell lines. Later it was named HER2 as it closes resembles the sequence of human epidermal growth factor receptor[25]. It contains a 185kD glycoprotein molecular component with tyrosine kinase activity.



MECHANISM OF HER2 GEME AMPLIFICATION MODEL

About 15-20% of the breast cancer cells demonstrate overexpression or HER 2 gene amplification. HER2 positive tumours benefit from taxanes and anthracycline based therapies, they are less responsive to other hormonal therapies. A humanised monoclonal antibody, trastuzumab is effective against HER2 gene expression and improves the survival, time of progression and response rate compared to other drugs[31].

As per the new guidelines formulated by ASCO/CAP all newly detected invasive breast carcinomas should be checked for HER2 status as they play an important role in further management of breast carcinoma[28].The prognostic value in breast carcinoma is dependent on the following factors

- Tumor size
- Type of tumor – histology
- Lymphatic involvement
- ER and PR status
- HER2 status
- Nuclear grade
- Mitotic activity

According to CAP guidelines the tissue is to be paraffin embedded and 10% neutral buffered formalin fixed for at least 6 hours. Over - fixed tissue can give difference in interpretation but under-fixed tissue usually gives false negative test[29]. Though different methods of fixation are available, each of them have to be compared with the standard protocol.

The antibodies CB11 and 4D5 are used to determine HER2 status. 4B5 a newly developed rabbit monoclonal antibody is available for better determination for HER2 status[30]. The HER2 IHC is interpreted as follows

- 0(negative) – no staining / < 10% membranous staining in tumor cells
- 1+(negative) – barely precipitated staining in >10% of tumor cells
- 2+(equivocal) – weak to moderate complete staining in > 10% of tumor cells
- 3+(positive) – strong complete membrane staining in >30% oftumor cell

All 2+ IHC cells are subjected to FISH for confirmation.

FISH in HER2/NEU

This technique uses molecular cytogenetics as basis and requires special fluorescent probes to detect DNA sequences in chromosomes. The probe identifies gene amplification. It comes as either single or dual coloured probes with sequences labelled for HER2 and the other for CEP 17 (chromosome 17 centromere)[29]. The single probe detects gene amplification, whereas the dual probe measures the ratio of HER2 to that of CEP17.

HER2 gene is unique in its gene amplification sequencing and is tightly bound to the protein which is characteristic to this in particular and differentiates it from other genes[30]. In case of dual probes which measures the ratio the cut off mark has been set by standard guidelines which include the following.

- <1.8 – negative
- 1.8 – 2.2 – equivocal
- >2.2 – positive

In case of single colour probe which determines the gene amplification the scores are fixed as following

- HER2 <4 – negative
- HER2 4-6 – equivocal
- HER2 >6 – Positive

Studies have proven that FISH is a better predictor of HER2 compared to IHC. The assay is to be preferably performed in resection specimens than core biopsies.

The Limitations of FISH include the following

- Morphological features are not distinct
- Need for dark field fluorescenct microscopy

CISH (chromogenic in situ hybridisation) makes use of DAB (diamino – benzidine)as chromogen for signalling and has gained recent popularity but still less accepted as it uses bot IHC as well as cytogenetic theories and has difficulty in interpretation[29,31].

In order to overcome the demerits of CISH, SISH (silver in situ hybridisation) is developed and it deposits silver ions to target cells by the use of enzyme linked probe and simplifies the procedure with easy identification of ions[32]. Owing to change in the modality of treatment

according to HER2 reports. HER2/NEU is considered to be the single most important theranostic predictor in breast carcinoma.

The other tumor markers employed in breast carcinoma include the following

- p53
- ki-67
- EGFR
- uPA and PAI- 1
- FOXA 1
- GATA 3

HER2 STATISTICAL DATA:

- In receptor status the single most important prognostic factor is the HER2 expression
- The HER2 positivity is usually high in high grade tumors[50]
- The HER2 positivity often shows a decrease in expression with advancing age[43]
- Overall 25 of all breast carcinomas overexpress HER2 irrespective of the stage of the disease[46,48]
- In recurrent metastatic breast carcinomas the conversion of HER2 positivity to negative status has been observed which denotes poor survival significance[51].
- As per CAP guidelines the recommended assay is FISH in case of HER2 2+ positive tumors by IHC[26]
- The surrogate classification of hormonal receptors is universally accepted combination pattern[48,49]
- As per the present data available no data had concluded that triple negative tumors have a similar survival pattern as compared to the other surrogate classification of hormonal receptors[48,50].

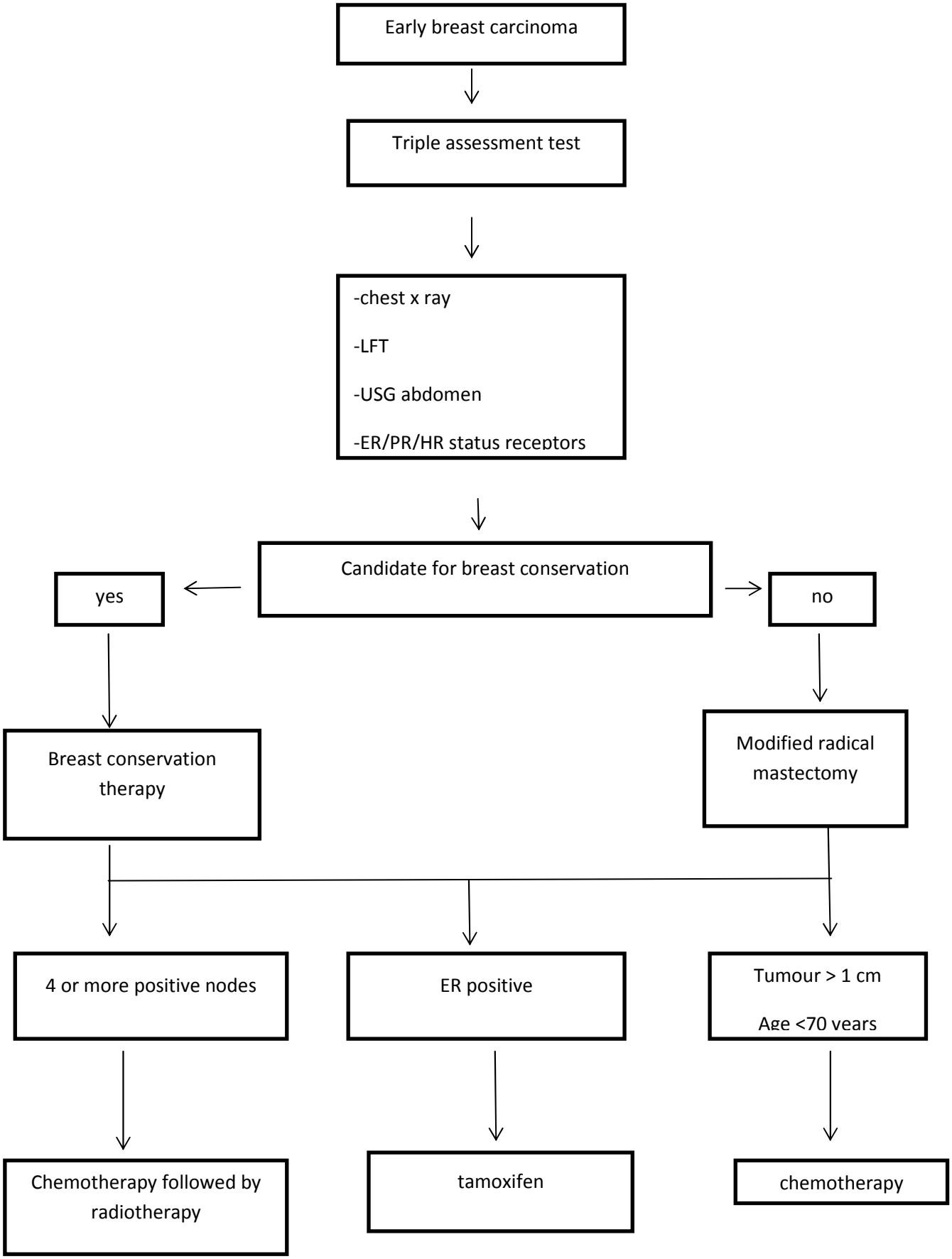
BRCA 1 AND 2:

BRCA 1 and BRCA 2 are located in the long arm of chromosome 17 and 13 respectively. They are considered to be cancer susceptible genes and are responsible for familial breast cancer. This tumor suppressor gene has autosomal dominant mode of inheritance and causes germ line mutation in the allele and predisposes every cell to cancer development[33].

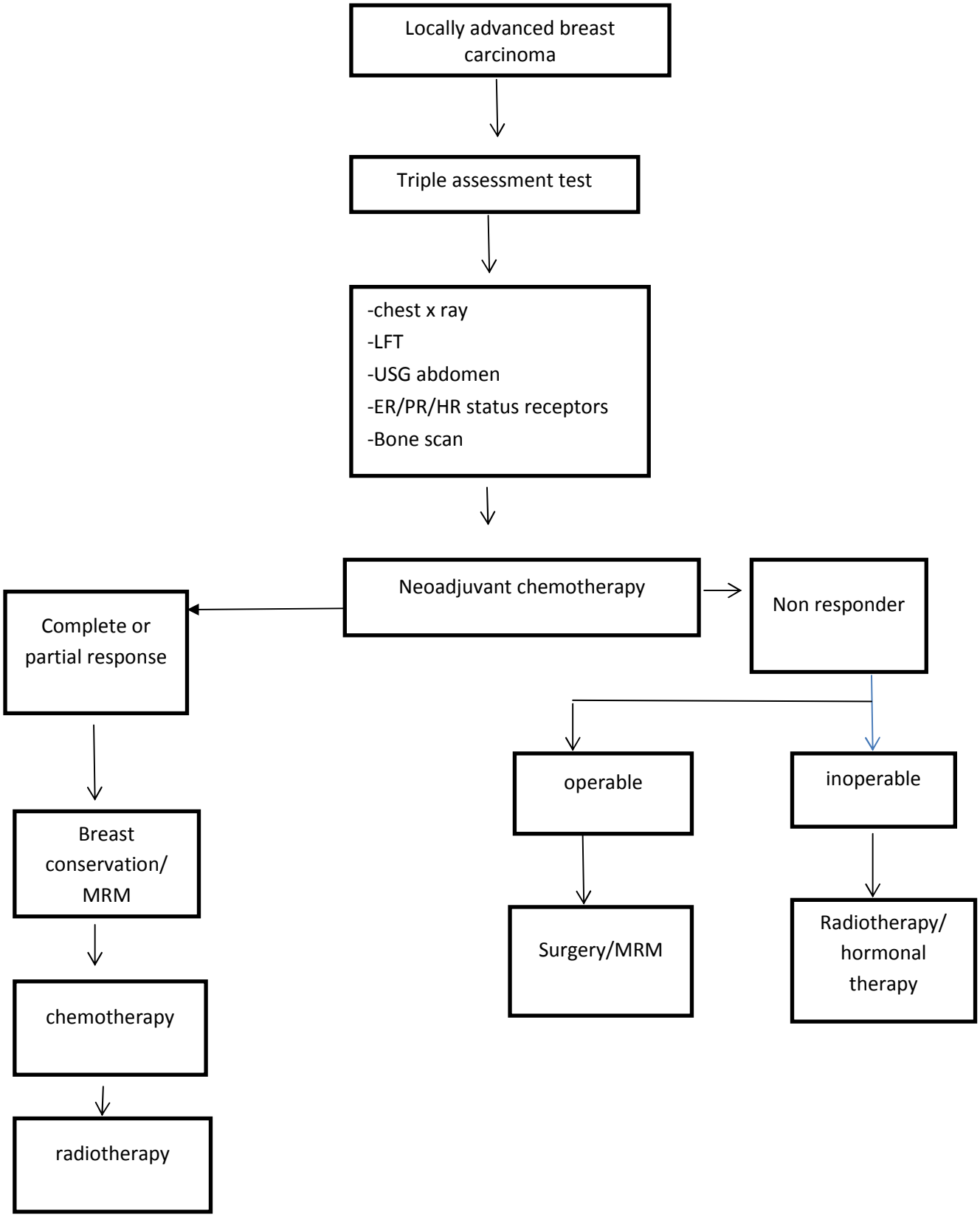
Unlike BRCA 1 the BRCA 2 predisposes the patient to ovarian malignancies. And any male breast carcinoma warrants a familial check on BRCA 2 as it predisposes to male breast carcinomas too. BRCA 2 positive tumors are usually HR negative, aneuploidy, high grade and show increased S phase fraction.

BRCA 1 and 2 positive patients are considered to be high risk in view of development of breast carcinoma and recent studies have proven prophylactic mastectomy to be effective and reduce the risk of by a great deal[33].

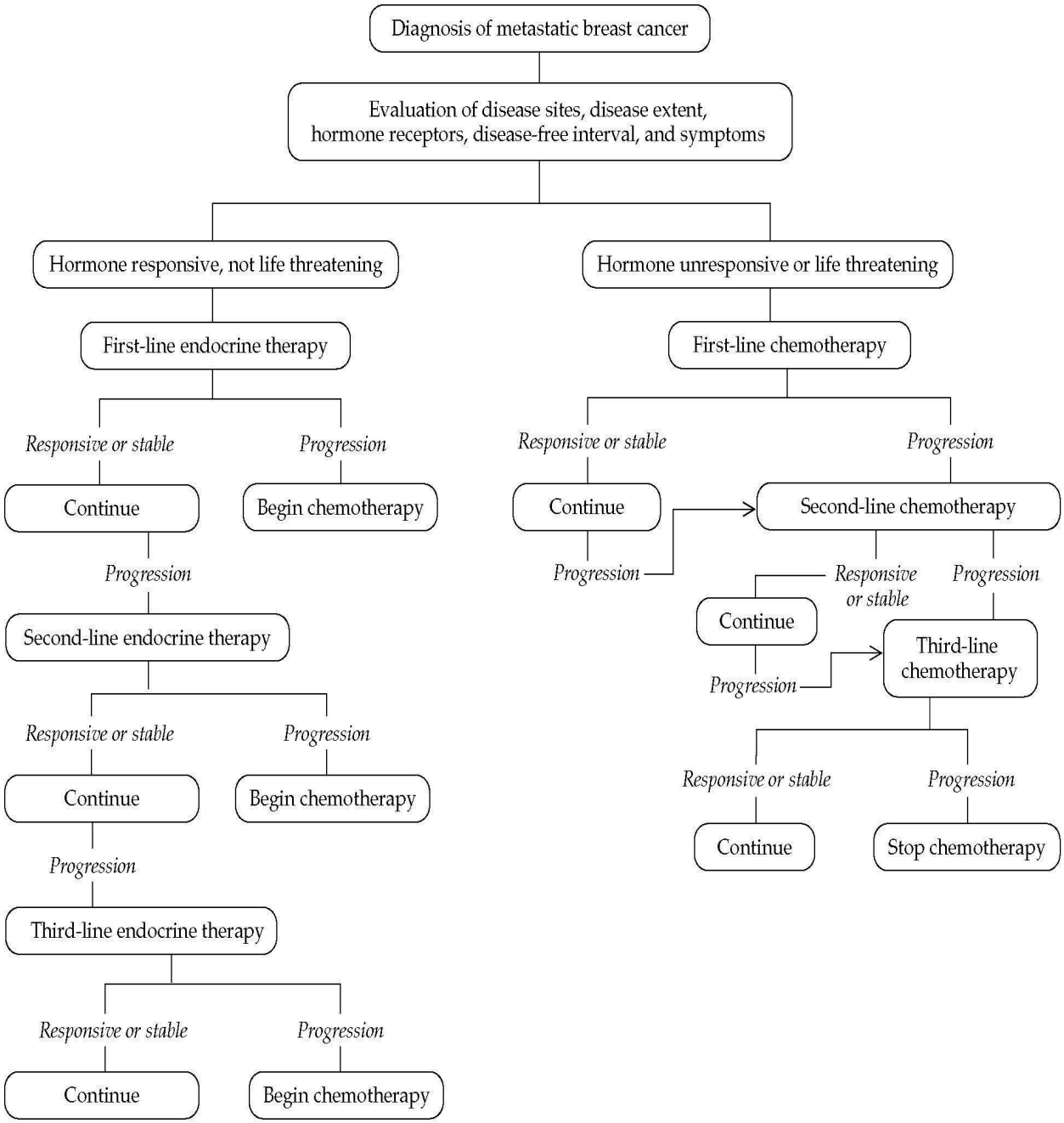
MANAGEMENT OF EARLY BREAST CARCINOMA



MANAGEMENT OF LOCALLY ADVANCED BREAST CARCINOMA



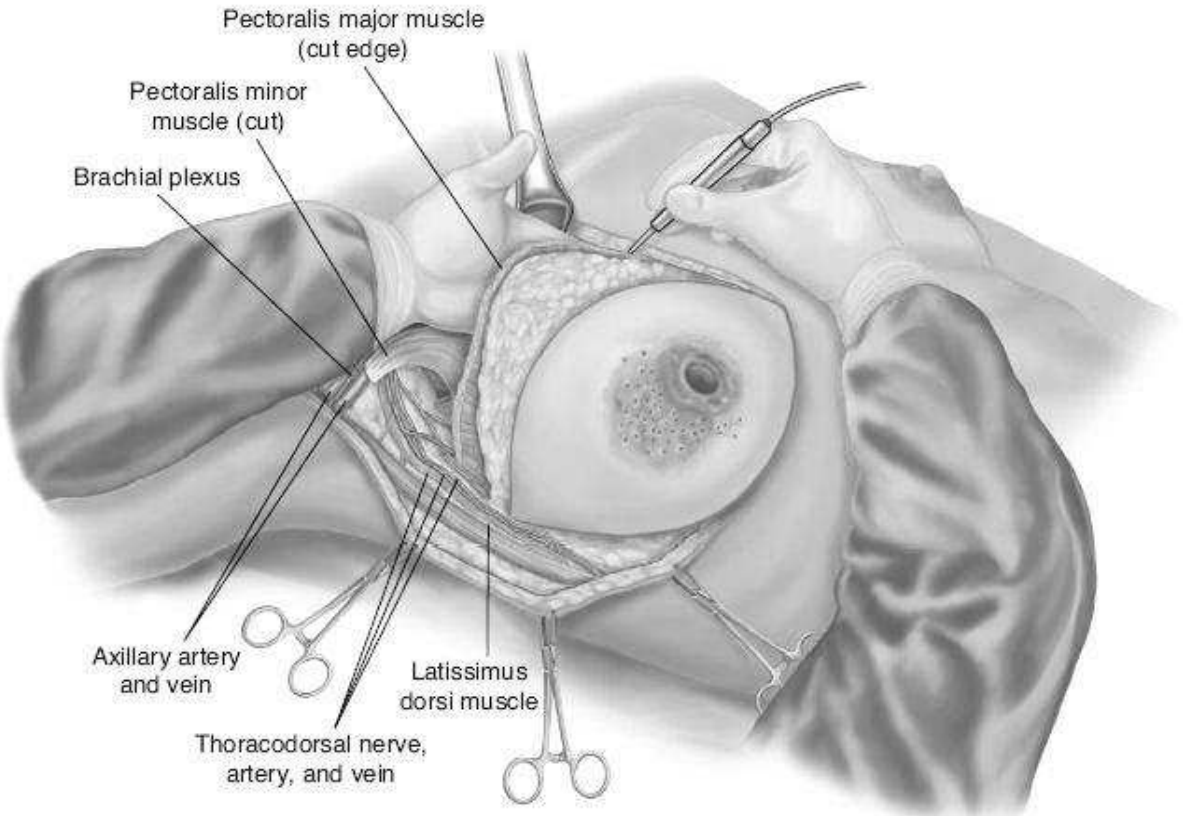
MANAGEMENT OF METASTATIC BREAST CARCINOMA



Modified radical mastectomy

In case of T1 - T3 lesions skin sparing mastectomy is performed in which the nipple areolar complex with the breast tissue and 1 cm of skin around the excised scar is removed. The simple mastectomy involves the removal of the nipple areolar complex, entire breast tissue and the necessary skin. Extended simple mastectomy involves the removal of level I lymph nodes along with the other structures listed in simple mastectomy. The modified radical mastectomy involves removal of all the breast tissue with nipple areolar complex, necessary skin and the level I and II lymph nodes[34].Patey modified MRM with removal of pectoralis minor muscle and level III lymph nodes. Halsted's mastectomy included removal of pectoralis major along with other structures, but current advances with radiotherapy and hormonal therapy, this concept is almost eliminated.

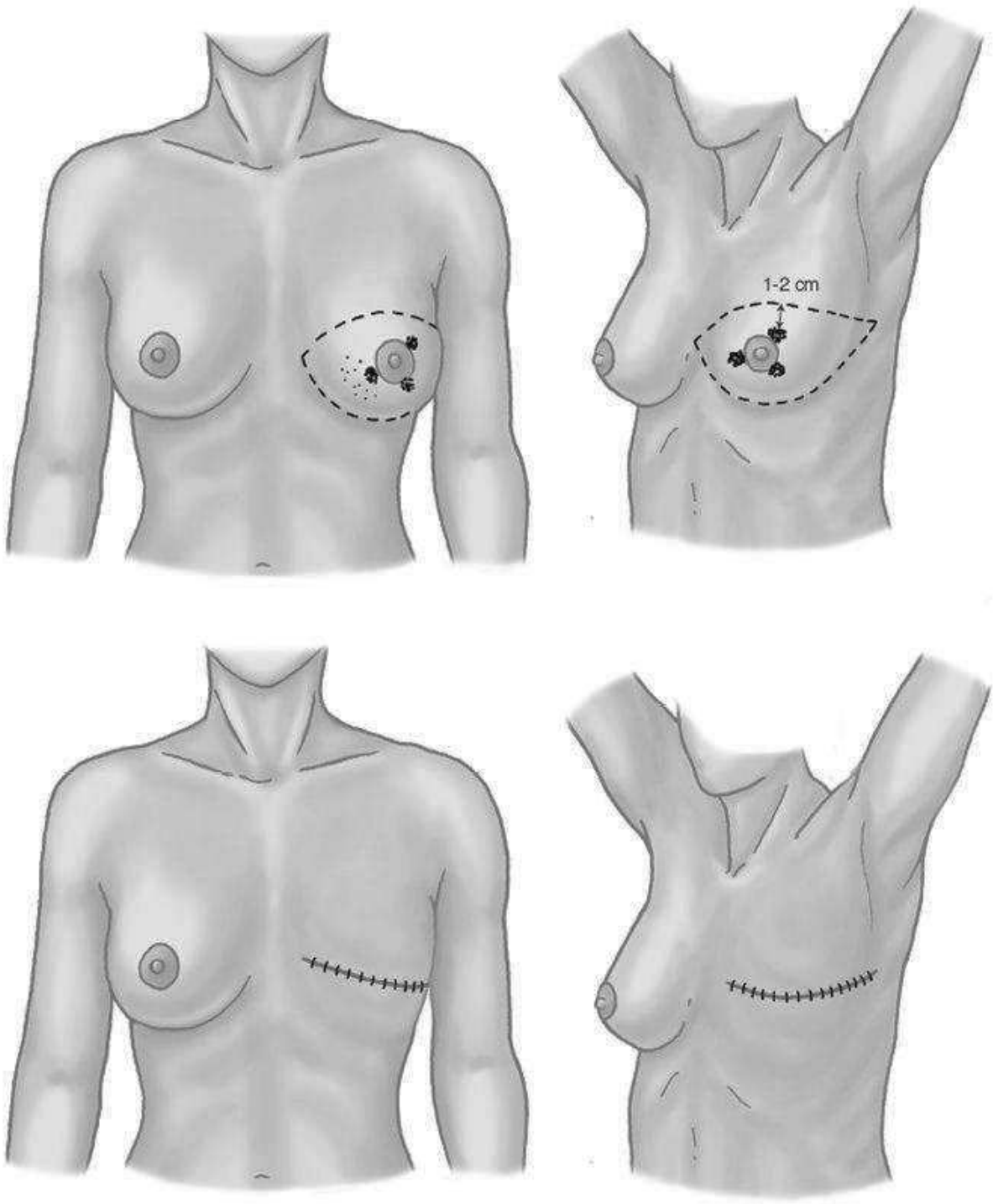
The patient is positioned supine with ipsilateral shoulder elevated in order to aid movements while performing the procedure. The ipsilateral arm is either fixed or rolled with stockinette to mobilise the arm during axillary dissection.



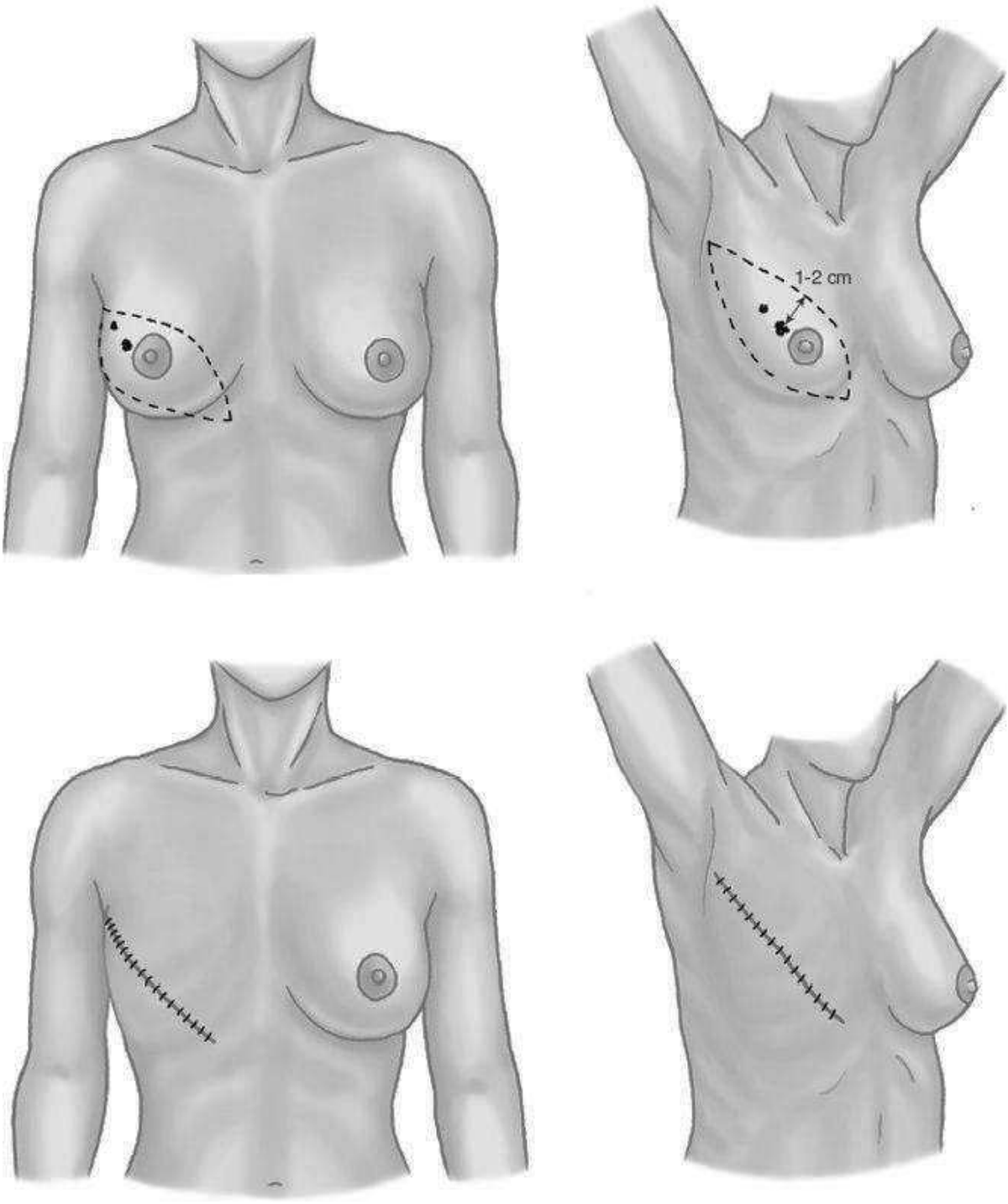
STRUCTURES TO BE PRESERVED IN MODIFIED RADICAL MASECTOMY

The skin incision is placed in such a way that the tumour is removed as an enbloc specimen with nipple areolar complex with overlying skin and skin margins clearance of 1 to 2 cm cephalad and caudad. The common incisions placed include the Stewarts and Orr's of which each has slight modifications to include adequate clearance[36].

STEWART'S INCISION



ORR’S OBLIQUE INCISION

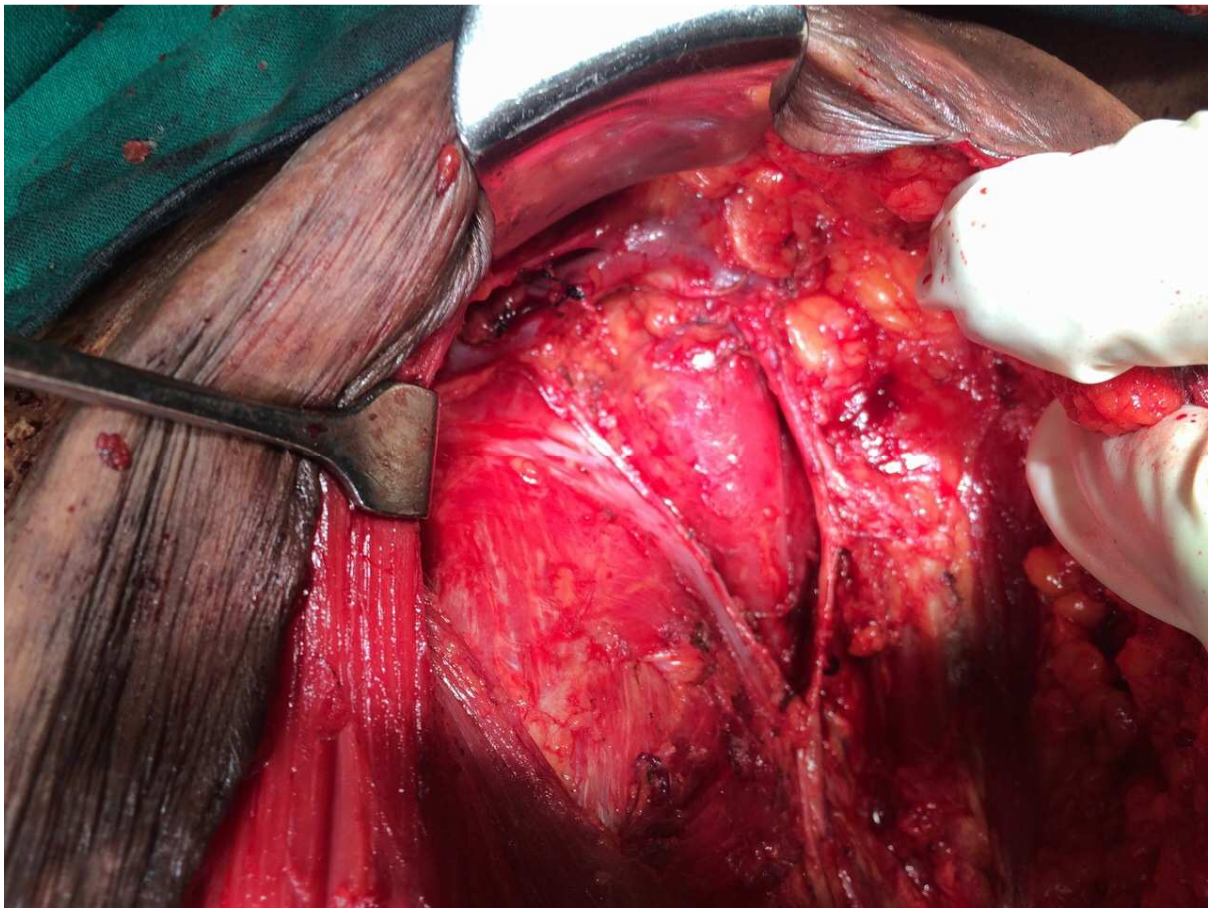


The skin flaps are raised exposing the anterior margin of latissimus dorsi laterally, sternum medially, subclavius muscle superiorly and rectus muscle inferiorly.The plane of dissection should be deep to the subcutaneous vasculature and superficial to the breast tissue in order to avoid flap necrosis and seroma formation. The breast tissue is dissected along with the pectoralis fascia starting from medial boundary until pectoralis minor is exposed. Care is taken not to injure the medial pectoral nerve as it causes atrophy of the pectoralis major muscle.

The axilla is entered by dividing the investing fascia and interpectoral

(rotter's nodes) lymph nodes are cleared. Later by dividing the loose areolar tissue the lateral extent of axillary vein lying antero-inferiorly to the axillary artery and brachial plexus is identified[35,36]. The fascia over the anteroventral surface of the axillary vein is sharply dissected exposing the level I and II lymph nodes.

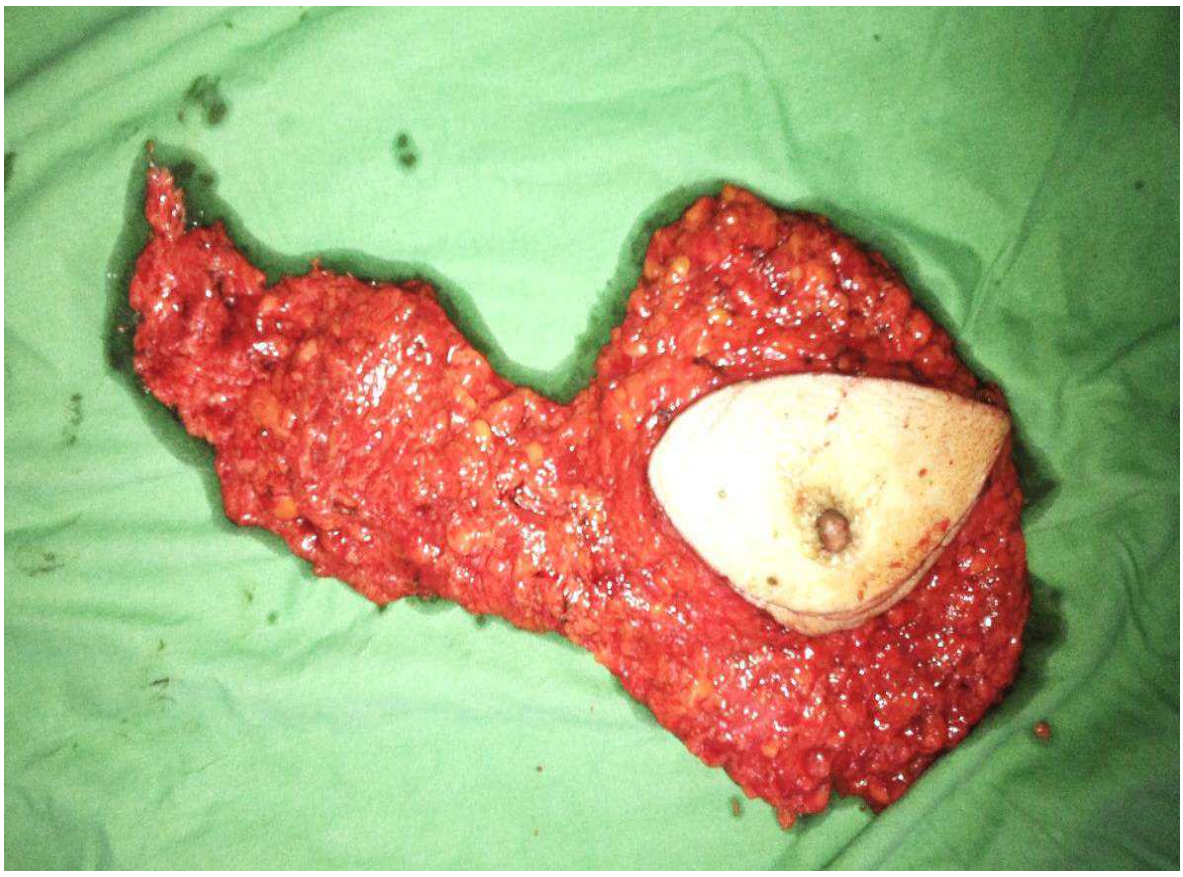
The thoracodorsal bundle is located deep in the axillary space and invested by loose areolar tissue. The thoracodorsal nerve originates from the posterior cord and supplies the latissimus dorsi muscle. The loose areolar tissue surrounding the thoracodorsal bundle is dissected by medial retraction of the lateral nodes and removed in enbloc along with subscapular group of lymph nodes[34,35,36].



INTROP PICTURE SHOWING ASILLARY DISSECTION

Dissection is further continued exposing the posterior contents of axilla and later medially removing the central group of lymph nodes. The superomedial limit of axillary dissection is marked with a stapler for pathological orientation. In the medial aspect of axilla, chest wall is exposed and nerve to serratus anterior is encountered, which lies in front of the subscapularis muscle.

In patey's modification the tendinous portion of the pectoralis muscle is removed to aid in clearance of level III lymph node. Thorough saline lavage is give and haemostasis is secured. Axillary and flap drains are placed separately and fixed. Skin is approximated in layers. Drains are retained till the collection is < 30ml/24 hours for 2 days.



MODIFIED RADICAL MASECTOMY SPECIMEN EXCISED IN TOTO

The possible complications post mastectomy include [36]

1. Seroma (30%)

2. Wound infection

3. Bleeding

4. Flap necrosis

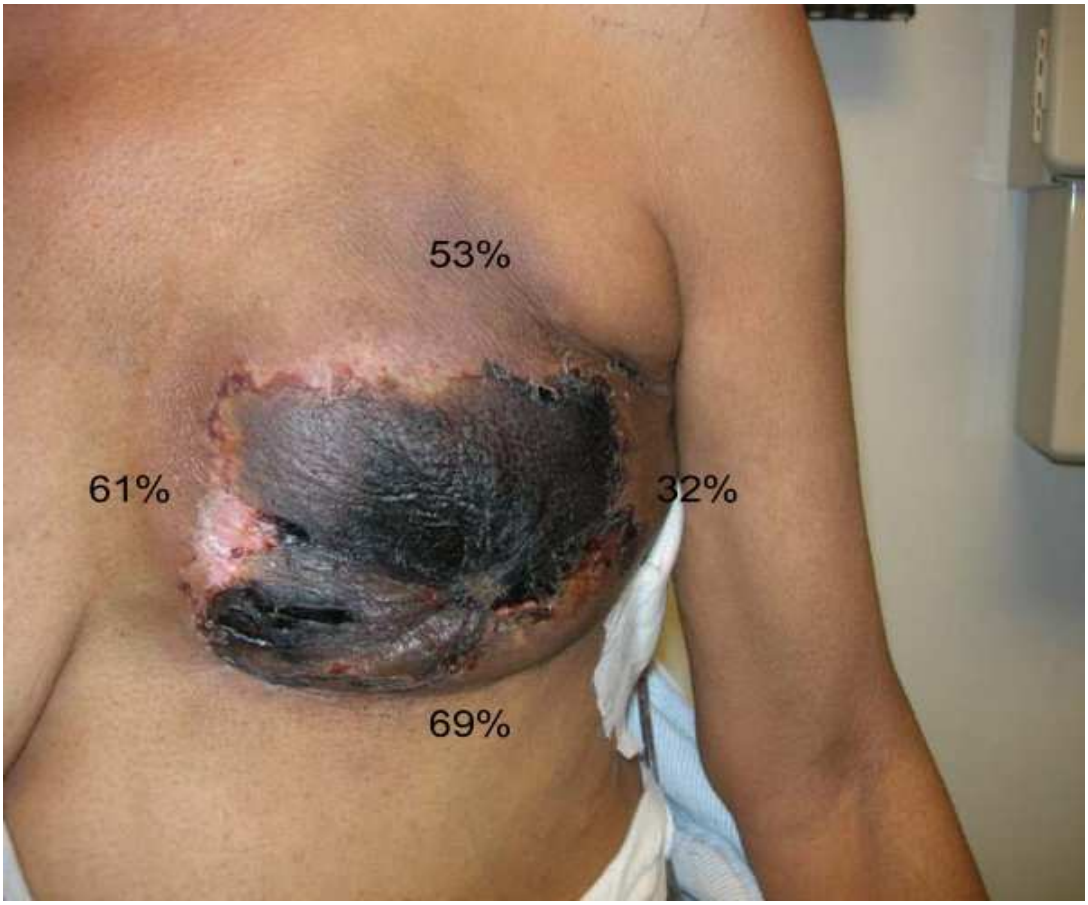
5. Lymphedema

6. Intercostobrachial nerve injury (numbness of medial aspect of upper arm)

7. Long thoracic nerve injury (winged scapula)

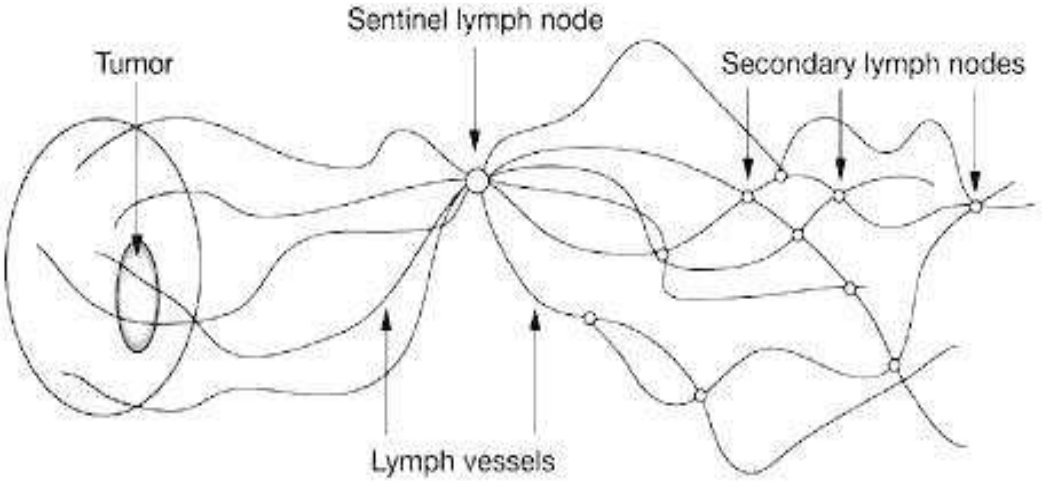
8. Medial and lateral thoracic nerve injury (Pectoralis muscle atrophy)

9. Thoracodorsal nerve injury (internal rotation and abduction of shoulder)



FLAP NECROSIS

SENTINEL LYMPH NODE BIOPSY



Sentinel lymph node is considered to be the first lump node encountered by the primary tumour, and owing to its histological status the lymph basin involvement of a metastatic disease can be predicted[37]. Intra-operatively the node is excised and sent for frozen section biopsy and its involvement is studied.

A clinically T1 or T2 disease without any lymphadenopathy warrants a sentinel lymph node biopsy before proceeding to axillary dissection. The agents used include technetium[99] labelled colloid albumin and isosulfan vital blue dye which is given either as subdermal / peritumoral / intratumoral injections.

The dye is given either the day prior to surgery or on table and the uptake is picked up by a gamma probe and frozen sectioning is done[37]. The advantages of sentinel lymph node biopsy include the following

- Cost effective
- Minimally invasive technique
- Micro-metastasis can be studied better
- Can avoid axillary dissection

The disadvantages include

- Skip metastasis is possible
- Not useful for stage IIB lesions

SERM

Selective estrogen receptor modulators have antagonistic and agonistic activity in different tissues in the body. They compete in occupying the receptor space in breasts and reduce the binding coefficient of estrogen in breast tissue. Meanwhile they have an agonistic action in uterine receptors. The main use of tamoxifen is its use in ER positive breast tumours and metastatic breast disease. Tamoxifen is orally absorbed and metabolised by CYP450 and is excreted in bile[38].

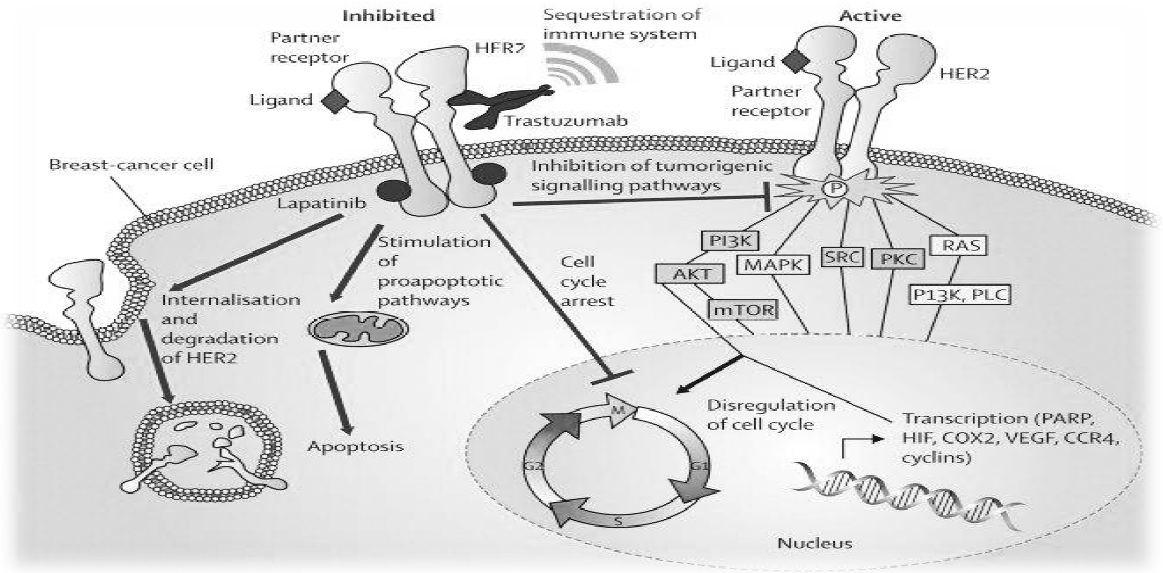
The adverse effects include hot flushes, nausea, vomiting and has the potential to develop endometrial cancer in long standing cases.

MECHANISM OF ATION OF SERM

TRASTUZUMAB

Around 25 - 30% of breast malignancies overexpress the HER2 receptor. Trastuzumab is a monoclonal antibody with DNA recombinant technology targeted specifically against these receptors. It decreases the S phase growth of tumour cells by inhibiting the cellular proliferation, the other possible hypothesis include antibody mediated cytotoxicity or down regulation by its effect on angiogenesis[39].

Trastuzumab is available as IV formulation and should be carefully administered in patient with cardiac dysfunction as it has a potency to develop heart failure. The cardiac complication of this drug is worsened if the drug is combined with anthracyclines[39].



MECHANISM OF ACTION OF TRASTUZUMAB

CHEMOTHERAPY

The indications for chemotherapy include the following.

- Locally advanced breast carcinoma and metastatic
- Node positive status
- Triple negative cases
- HER2 positive cases
- Size >0.5cm and less than 70 years of age

The new concept of breast malignancy is that it a systemic disease from the very beginning and in order to tackle the micrometastasis chemotherapy is indicated[40]. The common regimen used is cyclophosphamide, adriamycin and 5 -fluorouracil.

PAGET'S DISEASE OF THE NIPPLE:

This condition is associated with destruction of the nipple, as it is superficial involvement of intraductal carcinoma. It is a close mimicker of nipple eczema and needs to be differentiated by tissue biopsy. They contain paget's cells filled with mucopolysaccharides located in retepegs of epidermis.

They usually present with an underlying lump and more than 90% of them are proven to be invasive ductal carcinoma. This condition warrants modified radical mastectomy

PAGETS DISEASE OF THE NIPPLE

FOLLOW UP

The chance of recurrence is more within the first 5years of treatment for carcinoma breast and should be under follow up 20 years post treatment. As per international guidelines all patients who undergo treatment for breast carcinoma should be followed up as per this protocol

- Every 4 months for first 2 years
- Every 6 months for third to fifth year
- Every yearly thereafter

-Chest x-ray and mammogram are done yearly

-Bone scan, CT chest/ abdomen and brain are done if warranted clinically

MATERIALS AND METHODS

MATERIALS

A Prospective study conducted in PSG Institute of Medical Sciences and Research comparing the ER/PR/HER2 status in different stages of breast carcinoma in 30 patients

QUESTIONNAIRE

NAME:

AGE:

SEX:

IP NO:

OP NO:

DOA:

HISTORY (ONSET, DURATION AND SITE):

PAST/PERSONAL/MARITAL AND MENSTUAL HISTORY IF RELEVANT:

PHYSICAL EXAMINATION (SIZE, NODAL STATUS AND METASTATIC DISEASE FINDINGS):

MAMMOGRAM/USG BREAST:

FNAC/BIOPSY:

CXR,LFT,USG ABDOMEN & PELVIS:

MRI/PET (if any):

NEOADJUVANT CHEMOTHERAPY (if any)

SURGERY PLANNED:

HISTOPATHOLOGY REPORTS:

ER/PR/HRSTATUS:

INCLUSION CRITERIA:

- Women of all age groups
- Biopsy/FNAC proven Carcinoma Breast
- Bilateral involvement
- Any stage

EXCLUSION CRITERIA:

- Suspicious benign breast diseases
- Male breast carcinoma
- Phyllodestumour

METHODOLOGY:

In this study 30 patients were divided and sub – categorised into early, late and metastatic breast carcinoma after clinical examination, each containing a group of 10. Informed consent was obtained detailing the study process, need for the study and its benefits, elaborately. The history was recorded by the principal investigator and the mode of presentation (lump, pain, nipple discharge/retraction), duration and progression. Family and past history of any breast or gynaecological malignancies were enquired in detail.

All 30 patients were subjected to clinical examination by the principal investigator and to avoid bias the patient was re-examined by a faculty member and clinical TNM staging of the disease was formulated.

Triple assessment was done for all 30 patients and categorisation was confirmed naming them as early breast carcinoma, locally advanced breast carcinoma and metastatic breast carcinoma. They were subjected to either trucut biopsy or in case of FNAC proven malignancy – Modified radical mastectomy was done and resected specimen was assayed for ER/PR and HER2.

All patients underwent metastatic workup with liver function test, chest x ray, ultrasonography of the abdomen and in suspicious cases of metastatic disease the patients were subjected to PETCT/ MRI / Bone scan for further disease spread.

In all 30 patients Immunohistochemistry assay was carried out in the specimen in our NABH accredited lab and reported as per standard Allred scoring system and CAP guidelines. In case of HER 2+ status, FISH was not carried out due to non-availability.

The patients underwent either surgery, radiotherapy, hormonal therapy, neoadjuvant /adjuvant chemotherapy depending on the staging of the disease. All the above details elicited and investigated were uniformly

done in the same imaging and pathological set up to avoid bias. All the details are documented in a questionnaire format and confidentially preserved with the principal investigator of this study.

RESULTS AND OBSERVATIONS

OBSERVATION

ANALYSIS OF DATA

- Total of 30 cases were included in this study
- They were sub- categorised into early breast carcinoma, locally advanced breast carcinoma and metastatic breast carcinoma.
- As a prognostic indicator the various combinations of ER, PR and HER2 are categorised as follows[46]

GOOD

- ER+ PR+ Her2−
- ER− PR+ Her2−
- ER+ PR− Her2−

MODERATE

- ER+ PR+ Her2+
- ER− PR+ Her2+
- ER+ PR− Her2+

POOR

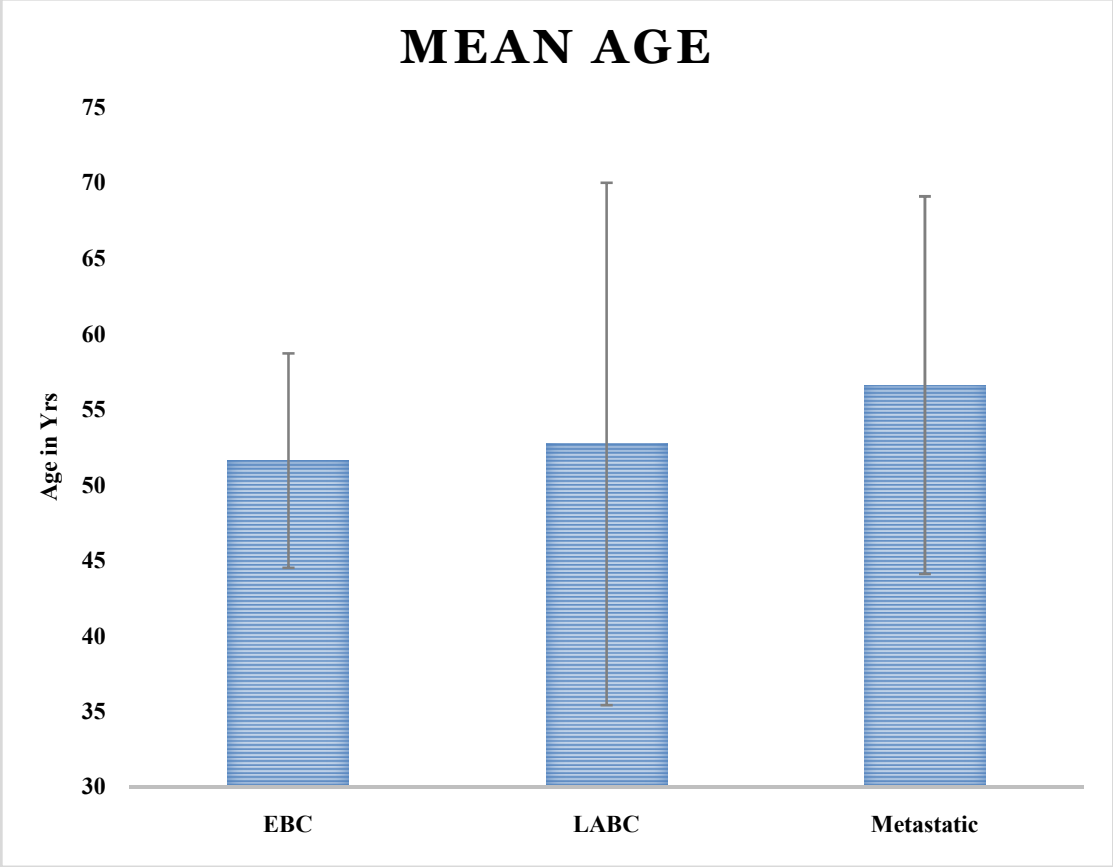
- ER− PR− Her2+
- ER− PR− Her2−

- All the 30 patients were systematically worked up with triple assessment and receptor status and were documented after obtaining informed consent.

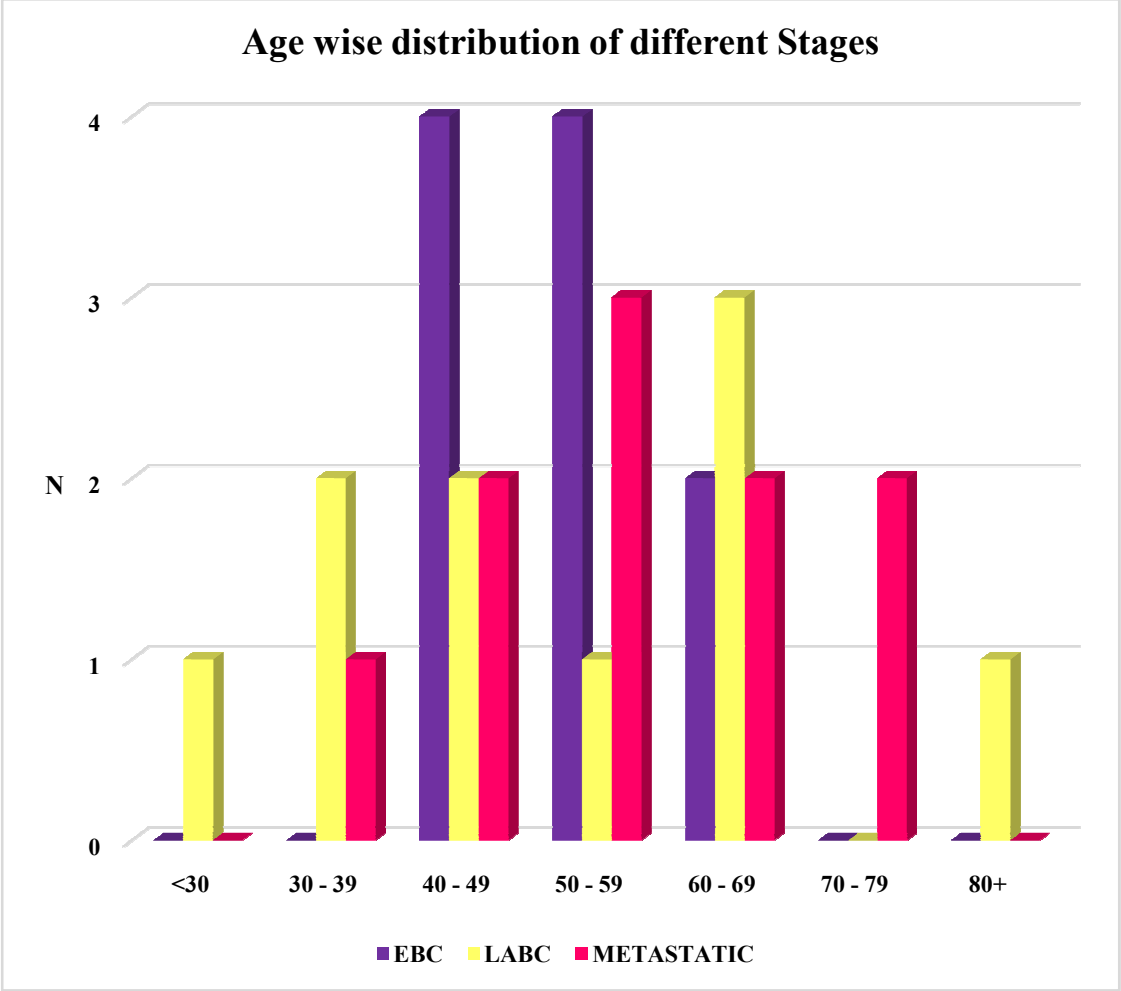
DATA ANALYSIS

Data collected were entered in Excel Spread sheet and analysed using STATA statistical software package release 11. We used the two-sided independent-samples t test to compare means across dichotomous variables (i.e. men v. women); the one-way ANOVA test for comparison of means across multilevel variables. Pearson's Correlation analysis was done and correlation coefficient was derived. Simple calculations like Percentages, Proportions and Mean values were derived. A type I error of 0.05 was considered in all analyses.

AGE DISTRIBUTION



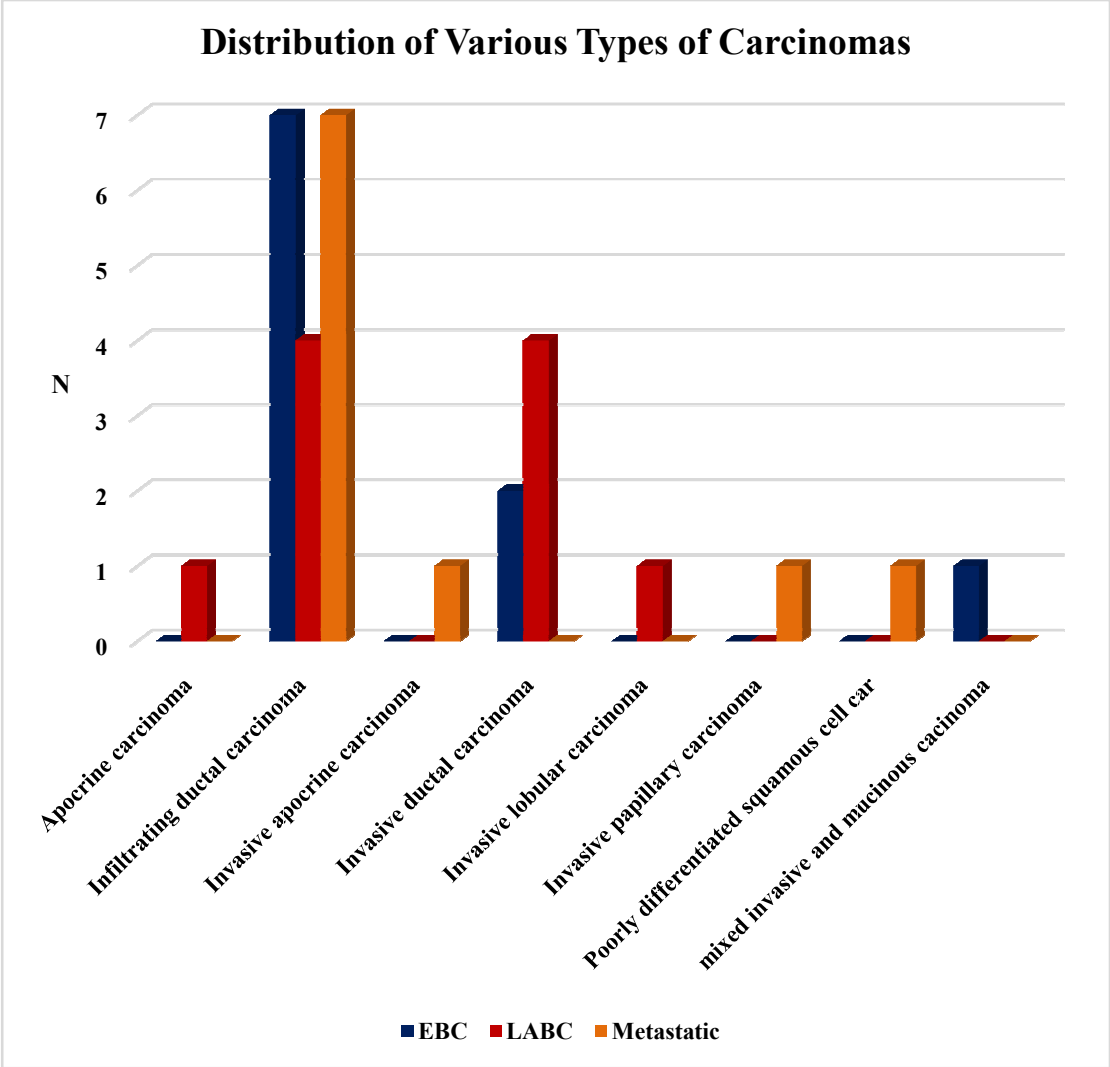
	EBC	LABC	Metastatic	P Value
Mean Age	51.6 ± 7.1	52.7 ± 17.3	56.6 ± 12.5	>0.05



Age in years	EBC	LABC	METASTATIC	Total
<30	0	1	0	1
30 - 39	0	2	1	3
40 - 49	4	2	2	8
50 - 59	4	1	3	8
60 - 69	2	3	2	7
70 - 79	0	0	2	2
80+	0	1	0	1
Total	10	10	10	30

- In this study the youngest patient was 28 years and the oldest patient was 82 years. The mean age was 53.6 years.
- Early breast carcinoma was predominantly occupying the 4^{th} to 6^{th} decade.
- Locally advanced breast carcinoma had a wide distribution of age group from the youngest to the oldest patient.
- Metastatic carcinoma shows an increasing trend as the age advances.
- More than 80% of the patients were between 40 to 70 years of age.

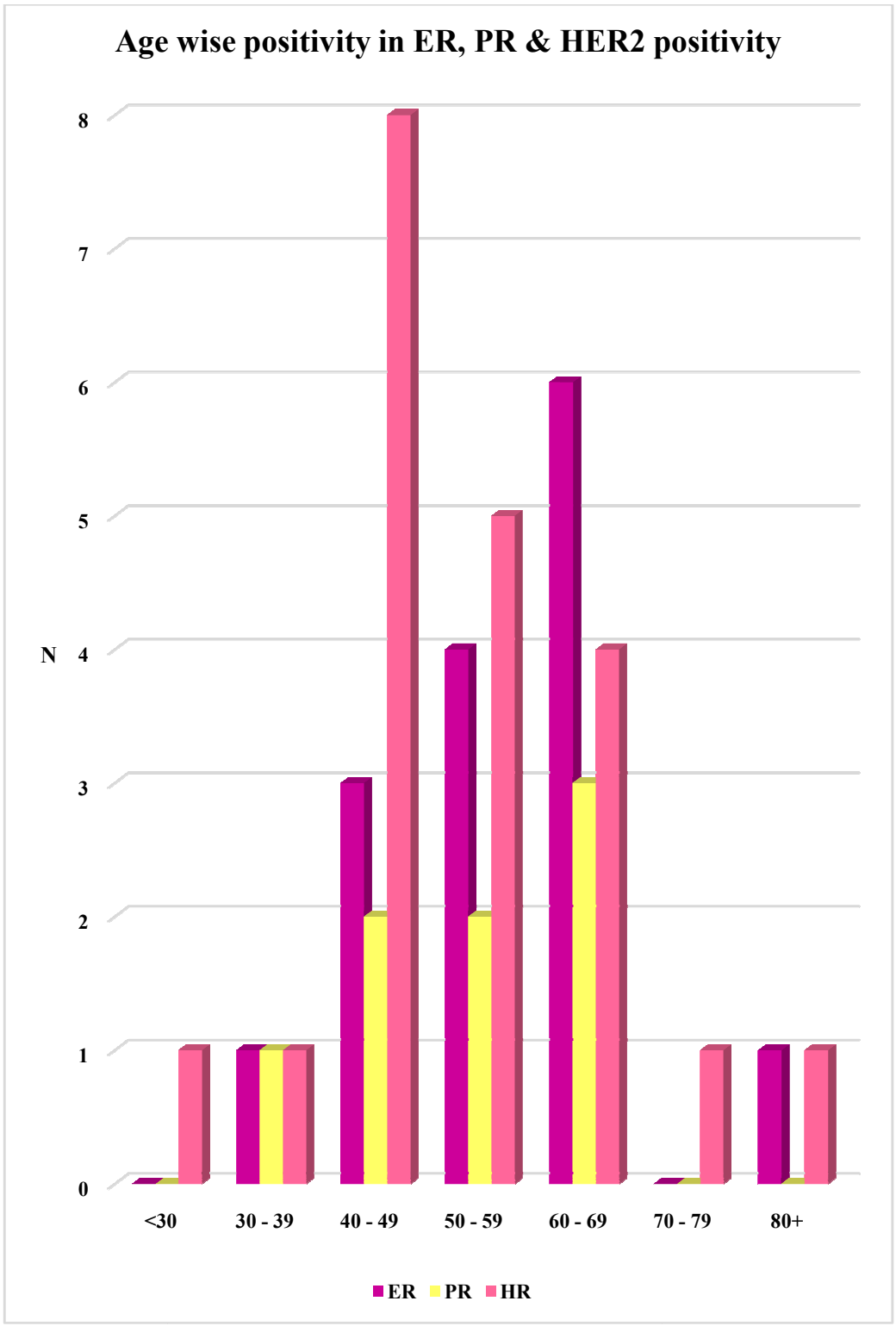
DISTRIBUTION OF TYPES OF CARCINOMA



Type of carcinoma	EBC	LABC	Metastatic	Total
Apocrine carcinoma	0	1	1	2
Infiltrating ductal carcinoma	9	8	7	24
Invasive lobular carcinoma	0	1	0	1
Invasive papillary carcinoma	0	0	1	1
Poorly differentiated squamous cell carcinoma	0	0	1	1
mixed invasive and mucinous carcinoma	1	0	0	1
Total	10	10	10	30

- The most common type of carcinoma is the infiltrating ductal carcinoma.
- The IDC occupies 80% of the study population.
- Predominant metastatic carcinomas also belong to IDC variety.
- A rare variety of poorly differentiated of squamous cell carcinoma of the breast is also encountered in our study which showed metastatic disease.
- One patient had mixed invasive and mucinous carcinoma.
- Medullary carcinoma, Colloid carcinoma, Tubular carcinoma, Cribriform carcinoma were not encountered in the study group.

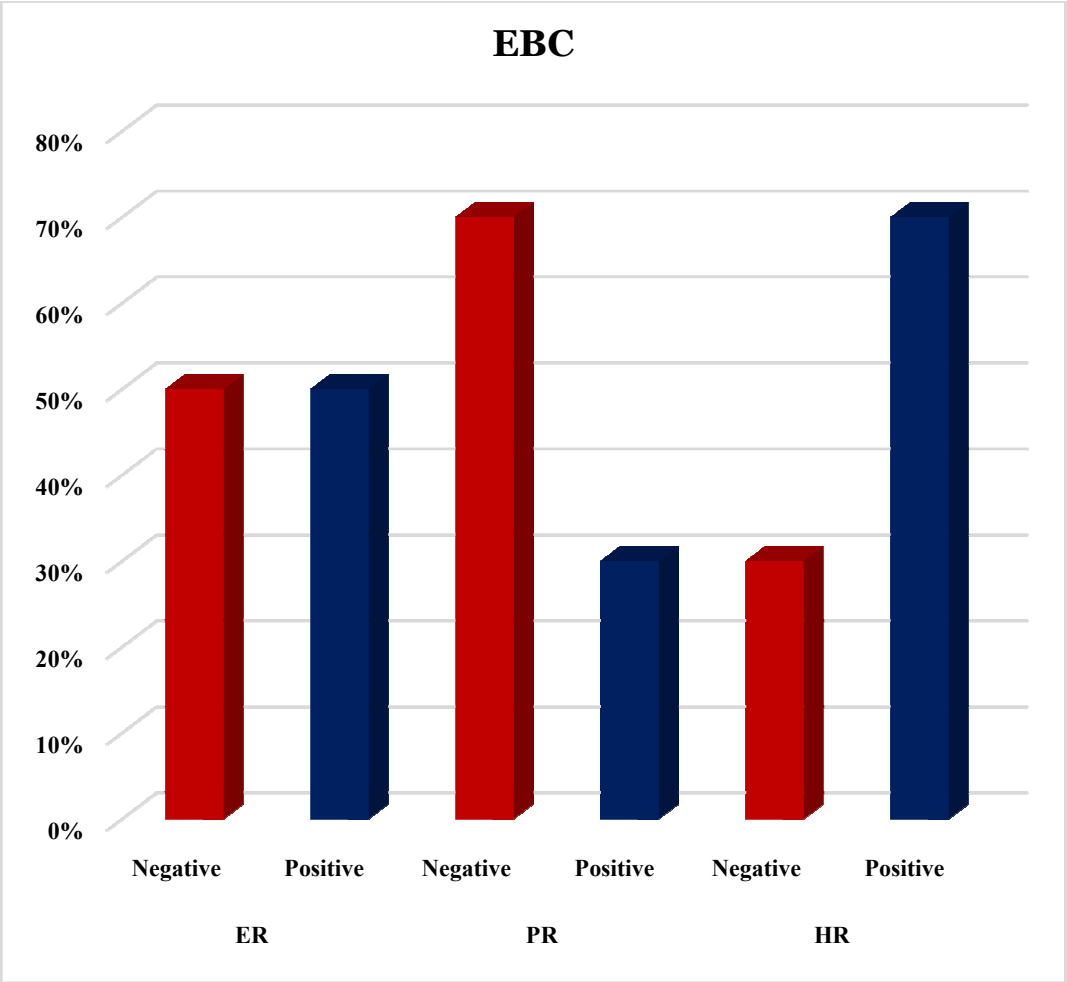
ER, PR AND HER2 STATUS


Age wise positivity in ER, PR & HER2 positivity
N
8
7
6
5
4
3
2
1
0
<30
30 - 39
40 - 49
50 - 59
60 - 69
70 - 79
80+
ER
PR
HR

Age in years	ER Positivity	PR Positivity	HER2 Positivity
<30	0	0	1
30 - 39	1	1	1
40 - 49	3	2	8
50 - 59	4	2	5
60 - 69	6	3	4
70 - 79	0	0	1
80+	1	0	1
Total	15	8	21

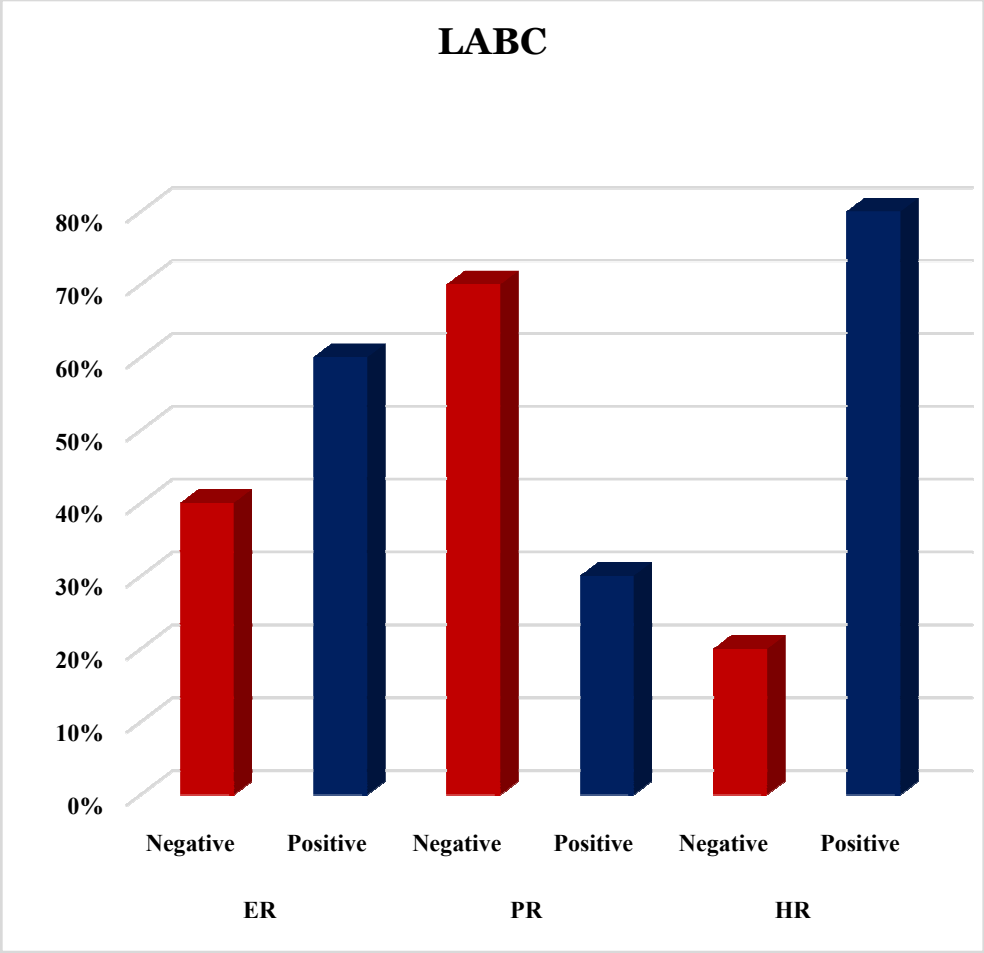
- Among 30 patient inclusive of all stages of carcinoma only 50% showed estrogen receptor positivity
- 70% of the study population showed HER2 positivity irrespective of the clinical staging.
- In this study group progesterone receptor positive status was seen only in 8 patients which is only 26.6%
- All 3 receptors namely ER/PR and HER2 showed their predominance in the age group between 40-70 years

EARLY BREAST CARCINOMA



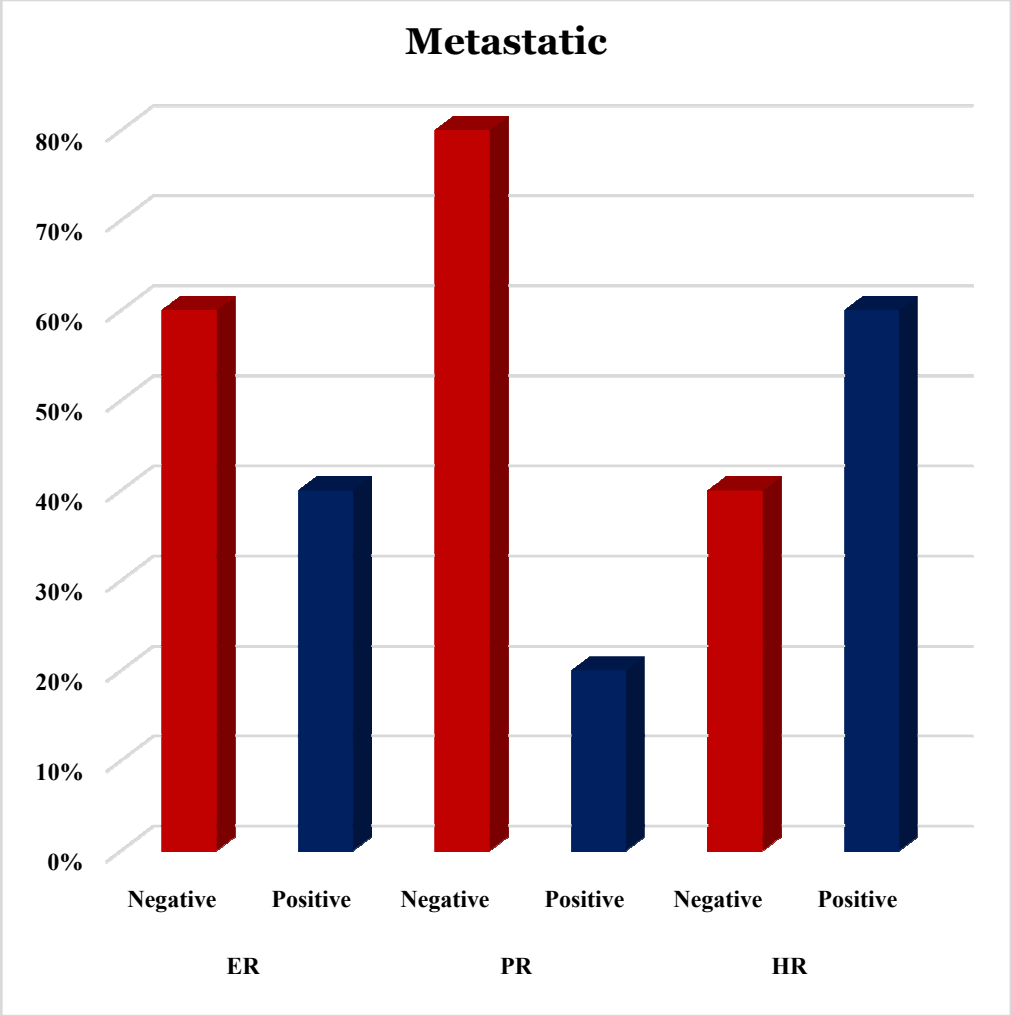
- In early breast carcinoma 50% were ER positive
- Only 30% showed PR positivity
- 70% of Early breast carcinomas showed HER2 positivity

LOCALLY ADVANCED BREAST CARCINOMA



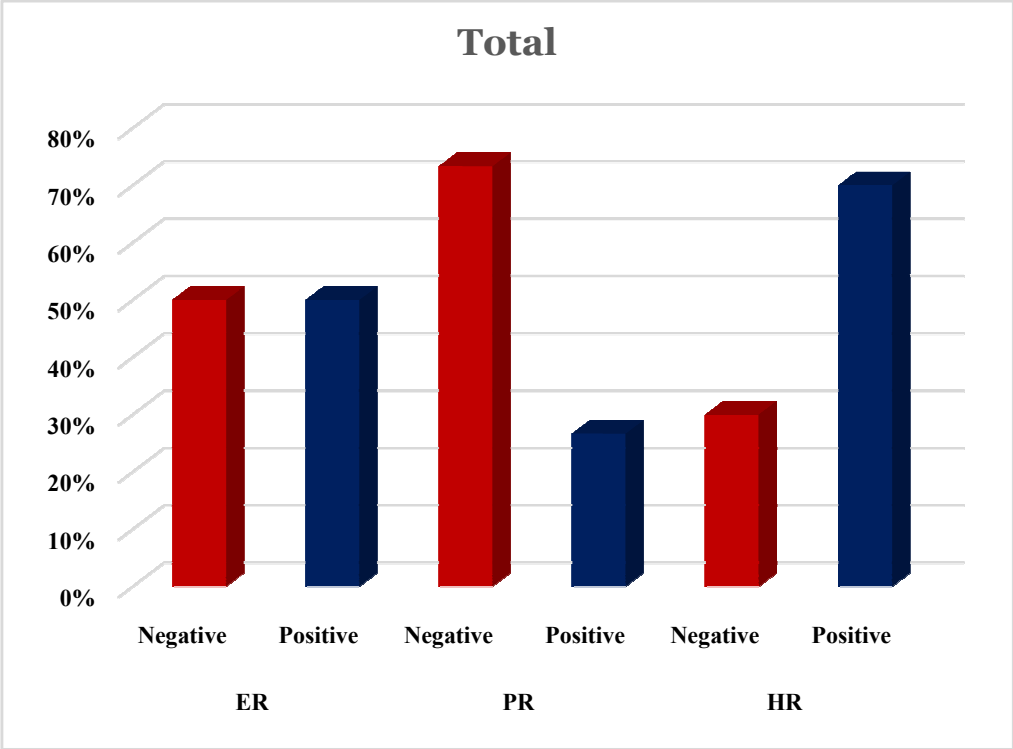
- 60% of LAB carcinomas showed ER positivity
- PR was positive only in 30% of Locally advanced breast carcinomas
- Only 2 patients were negative for HER2 in Locally advanced breast carcinomas

METASTATIC BREAST CARCINOMAS



- 40% of the metastatic tumors were ER positive
- Only 20% of metastatic breast carcinomas exhibited PR positivity
- HER2 was significantly positive in 60% of this study population

COMBINED STATISTICS

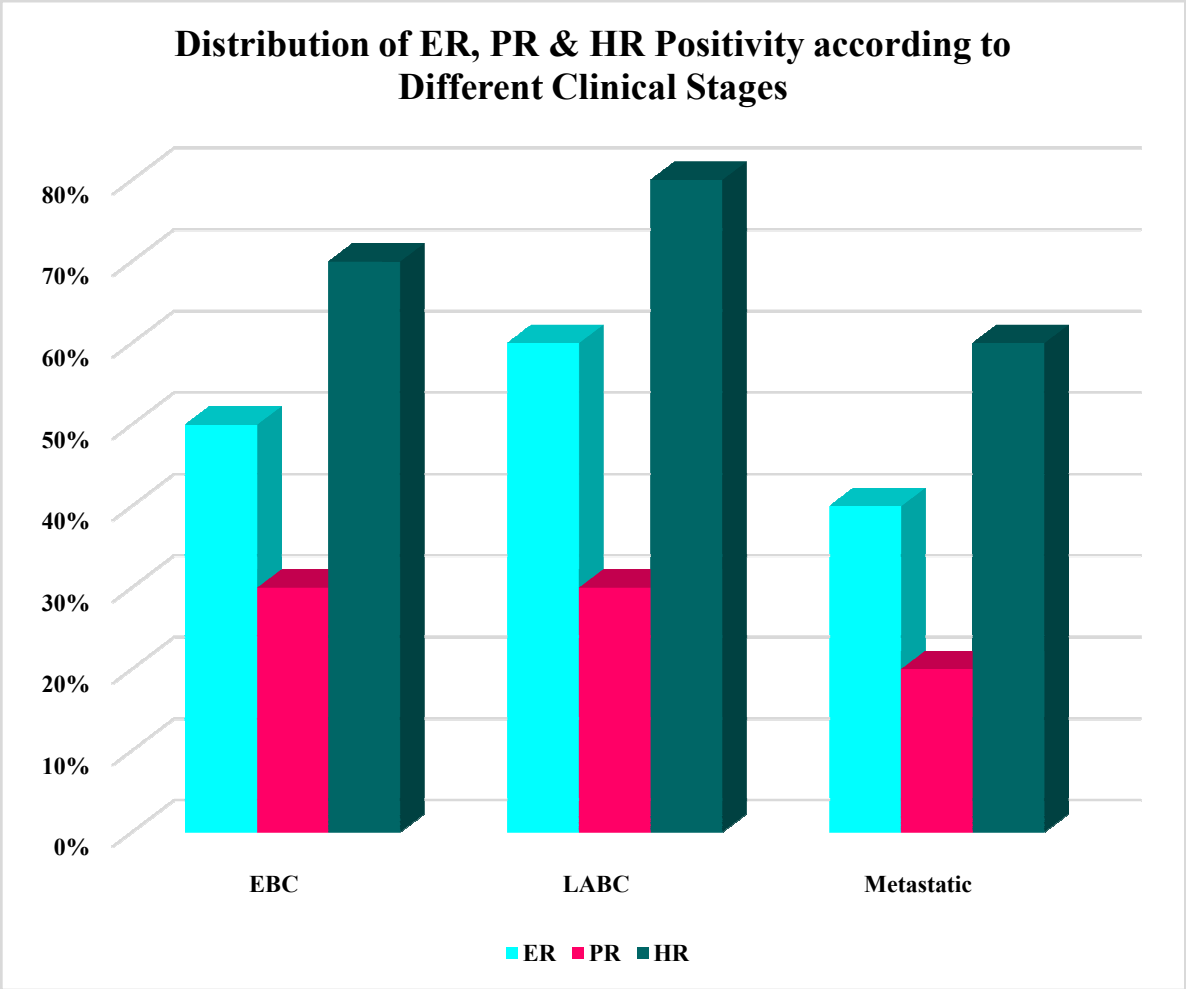


INCLUSIVE OF 3 DIFFERENT STAGES

	ER		PR		HER	
	Negative	Positive	Negative	Positive	Negative	Positive
EBC	50%	50%	70%	30%	30%	70%
LABC	40%	60%	70%	30%	20%	80%
Metastatic	60%	40%	80%	20%	40%	60%
Total	50%	50%	73.33%	26.67%	30%	70%

CORRELATION BETWEEN DIFFERENT RECEPTORS

	ER	**PR**	**HER**	**ER & PR**	**ER & HER**	**PR & HER**
	Positive	Positive	Positive	P Value	P Value	P Value
EBC	50%	30%	70%	>0.05	>0.05	>0.05
LABC	60%	30%	80%	>0.05	>0.05	<0.05
Metastatic	40%	20%	60%	>0.05	>0.05	>0.05
Total	50%	26.67%	70%			



The combined statistical data analysis of all 30 in the study group show the following

- Overall 50% of patients have ER positivity
- PR positivity is seen only in 26.67% of the study population
- HER is positive in 70% of the study population

- The p value of comparison of different results with one another show significance for comparison of PR and HER2 status in locally advanced carcinomas.

As mentioned above after categorising the ER,PR and HER2 prognostic significance and combination as good, moderate and poor in different stages the results obtained are as follows

stage	Hormonal prognostic predictors		
	Good	moderate	poor
EBC	5	5	0
LABC	6	3	1
Metastatic	4	3	3

- Early breast carcinoma has no patients in worst prognostic category of hormonal receptors
- In Locally advanced breast carcinoma 10% of the population fall under bad prognostic category
- 30% of metastatic carcinomas have bad prognostic index category
- Overall 50% of the patients fall under good hormonal prognostic index irrespective of the stage of disease
- 11 out of 30 patients fall under moderate prognostic criteria
- Only 13.3% of patients fall under poor prognosis category
- All 3 patients in metastatic disease with poor prognostic predictor had triple negative results

DISCUSSION

A prospective case study of estrogen, progesterone and Human epidermal growth factor 2 receptor status in different stages of breast carcinoma was studied in 30 patients and the study infers the following

This study expands the information available from prior research by assessing breast cancer staging with that of ER/PR/HER2 subtypes and we have concluded our experience in the study population which exhibits particular similarities and differences compared to that of international research data available

- The most common type of malignancy in the study group is invasive ductal carcinoma which is on comparison with international data[20]
- The mean age of the study group is 53.6 and is almost equal to the study conducted in south-indian population[43].
- 80% of the study population lie within 40 – 70 years age group and more in the peri-menopausal age group[43,44]
- The p value comparison of different results with one another show significance for comparison of PR and HER2 status in locally advanced carcinomas

- None of the early breast carcinomas showed poor hormonal prognostic index and is in disjunction compared to international studies[46,48].
- 30% of metastatic population had associated poor hormonal prognostic predictor and is correlating with the previous data available[46,47]
- HER2 was positive in 70% of the population which outnumbered the other hormonal receptors, whereas only 25% of the breast carcinomas overexpress HER2 universally[50]
- There is no specific pattern of hormonal receptors association in any of the particular stage which is different from the data available as most of the early breast carcinomas express ER and PR positivity[44]
- The overall progesterone receptor status positivity is only 26.66% which is far less compared to usual pattern in Indian population which is around 63%[42,43]
- There is no significant coexisting positivity status between estrogen receptor and progesterone receptor which is unusual as they usually express similarity pattern[48,49]

- All 3 patients (10% overall) in metastatic group had triple negative disease which is in conjunction with international data[46,47,50]
- Correlation Model between prognosis according to clinical and hormonal staging shows a positive correlation with correlation coefficient of 0.2659 with significance of >0.05 (overall)

CONCLUSION

CONCLUSION AND RECOMMENDATIONS

Hormonal therapy plays an important role in the management of breast carcinomas in the recent era and adds on to benefit the patient. It is also an important prognostic index for the disease progression and is now mandatory that all diagnosed patients of breast carcinoma should undergo hormonal receptor assay.

This studyshows the following similarities and variations compared to international data available.In our study ER reactivity was seen 50% and PR reactivity 26.67% of breast cancers whereas in a similar study conducted at the Liaquat national hospital, Karachi, Fatima et al (2005) showed 55% ER and PR reactivity.In this study we experienced 70% positivity which is grossly high compared to international data which shows only 25% positivity. Out of the four triple negative tumors encountered in this study 3 were belonging to metastatic group and all were histologically high grade which is in conjunction with the international data[50]. There is usually a coexisting pattern of expression between ER and PR which is not observed in this study[49].In early breast carcinomas the HER2 positivity is usually less but in our study we have experienced 70% positivity which is significantly high.

The study does have its own limitations due to a small sample number, selective sampling, interpreter variation and non-availability of FISH technique, but the limitation is overcome by extensive comparison standards and correlation with international data.

Herewith we conclude our overall experience studying the estrogen, progesterone and HER2 receptor in different stages of breast carcinoma in 30 patients and the study shows a significant positive correlation between the hormonal status and clinical staging overall.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Quantifying the natural history of breast cancer K H X Tan, L Simonella, H L Wee, A Roellin, Y-W Lim, W-Y Lim, K S Chia, M Hartman and A R Cook British Journal of Cancer (2013) 109, 2035–2043.
2. Early Signs and Symptoms of Breast Cancer Henry P. Leis, Jr, MD; West J Med. Sep 1980; 133(3): 225
3. Triple assessment in the diagnosis of breast cancer in Kashmir MasoodaJan, Javeed Ahmad Mattoo, Nazir Ahmad Salroo, and ShahnawazAhangar; Indian J Surg. Apr 2010; 72(2): 97–103.
4. The treatment of early breast cancer in women over the age of 70;A Ring, M Reed, R Leonard, I Kunkler, H Muss, H Wildiers, L Fallowfield, A Jones and R Coleman; British Journal of Cancer (2011) 105, 189–193.
5. The book "Diseases of the Breast 3rd Edition" is written by Monica Morrow.
6. The Breast: Comprehensive Management of Benign and Malignant Diseases, 4th Edition, by Kirby I. Bland, MD, and Edward M. Copeland, III, MD,

7. The Breast Cancer Alternative Hypothesis: Is There Evidence to Justify Replacing It? Bernard Fisher and Stewart J. Anderson.
8. Reconstruction of the Breast Conservation Deformity ;Sumner A. Slavin, M.D., F.A.C.S. and Terri Halperin, M.D. SeminPlast Surg. May 2004; 18(2): 89–96.
9. Social and ethical implications of BRCA testing;A. Surbone;AnnOncol (2011) 22 (suppl 1):i60-i66.
10. Anatomy of the lactating human breast redefined with ultrasound imaging; DT Ramsay, JC Kent, RA Hartmann, and PE Hartman; J Anat. Jun 2005; 206(6): 525–534.
11. Clinical Anatomy by Regions;Richard S. SnellLippincott Williams & Wilkins, 2008.
12. Gray, Henry & Carter, Henry Vandyke Anatomy Descriptive and Surgical, John W. Parker and Son.
13. The Lymphatic Anatomy of the Breast and its Implications for Sentinel Lymph Node Biopsy: A Human Cadaver Study; G. Bruce Mann, MB BS, Ph.D. FRACS, and G. Ian Taylor, A.O., M.D., F.R.A.C.S.; Ann SurgOncol. Mar 2008; 15(3): 863–871.
14. Triple assessment in the diagnosis of breast cancer in Kashmir Masooda Jan, Javeed Ahmad Mattoo,Nazir Ahmad Salroo,and ShahnawazAhangar; Indian J Surg. Apr 2010; 72(2): 97–103.

15. American Joint Committee on Cancer; 7th edition

16. Role of Imaging Techniques in Diagnosis of Breast Cancergheonea ioana andreer, raluca pegza, luana lascu, simona bondari, zoia stoica, a. bondari; Vol.37, No.2, 2011.

17. Detection of Breast Cancer With Addition of Annual Screening Ultrasound or a Single Screening MRI to Mammography in Women With Elevated Breast Cancer Risk; Wendie A. Berg , MD, PhD; Zheng Zhang, PhD; Dione M. Farria, MD; Jean B. Cormack, PhD; Helga S. Marques, MS; Amanda Adams, MPH; Nolin M. Yeh, MS; Glenna Gabrielli, BS; JAMA. 2012;307(13):1394-1404.

18. Diagnostic value of (18)F-FDG PET/CT and MRI in predicting the clinicopathologic subtypes of invasive breast cancer; Miyake KK[1], Nakamoto Y, Kanao S, Tanaka S, Sugie T, Mikami Y, Toi M, Togashi K. 2014 Aug;203(2):272-9

19. The utilization and impact of core needle biopsy diagnosis on breast cancer outcomes in British Columbia; Issue: BCMJ; Kristy Cho, Scott Tyldesley, MD, FRCPC, Caroline Speers, BA, Barbara Poole Lane, MPA, Karen A. Gelmon, MD, FRCPC, Christine Wilson, MD, FRCPC; Vol. 56, No. 4, May 2014, page(s) 183-190 Articles.

20. The current state of breast cancer classification;G.Viale*;Volume 23, Issue suppl 10.

21. Estrogen Receptor as an Independent Prognostic Factor for Early Recurrence in Breast Cancer William A. Knight III, Robert B. Livingston, Ernest J. Gregory, and William L. McGuire; 1977 American Association for Cancer Research.

22. Gene Expression and Benefit of Chemotherapy in Women With Node-Negative, Estrogen Receptor–Positive Breast Cancer Soonmyung Paik, Gong Tang, Steven Shak, Chungyeul Kim, Joffre Baker, Wanseop Kim, Joseph P. Costantino, Charles E. GeyerJr,; 2006 by American Society of Clinical Oncology.

23. Estrogen Receptor Status by Immunohistochemistry Is Superior to the Ligand-Binding Assay for Predicting Response to Adjuvant Endocrine Therapy in Breast CancerJennet M. Harvey, Gary M. Clark, C. Kent Osborne and D. Craig Allred.

24. Tumor Microenvironment of Metastasis and Risk of Distant Metastasis of Breast CancerJNCI J Natl Cancer Inst Aug 1, 2014:dju136.

25. Past, Present, and Future Challenges in Breast Cancer Treatment; JCO Jul 1, 2014:1979-1986.

26. Prediction of benefit from chemotherapy in ER-positive/HER2-negative breast cancerAnn Oncol Mar 1, 2014:755

27. Mechanisms of Tamoxifen Resistance: Increased Estrogen Receptor-HER2/neu Cross-Talk in ER/HER2–Positive Breast

Cancer; Jiang Shou, Suleiman Massarweh, C. Kent Osborne, Alan E. Wakeling, SimaleAli, Heidi Weiss and Rachel Schiff ; JNCI J Natl Cancer Inst,Volume 96, Issue 12Pp. 926-935.

28. Prevalence of amplification of the oncogenes c-myc, HER2/neu, andint-2 in one thousand human breast tumours: Correlation with steroid receptors Els M.J.J. Berns, Jan G.M. Klijn, Iris L. van Staveren, HenkPortengen, Erica Noordegraaf, John A. Foekens; Breast Cancer Working Conference, 3–6 September 1991, Leuven.

29. Expression of p95HER2, a Truncated Form of the HER2 Receptor, and Response to Anti-HER2 Therapies in Breast Cancer Maurizio Scaltriti, Federico Rojo, JNCI J Natl Cancer InstVolume 99, Issue 8Pp. 628-638.

30. HER2-Positive Breast Cancer; Ryan H. Engel, Dr Virginia G. Kaklamani.

31. Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2;Dennis J. Slamon, M.D., Ph.D., Brian Leyland-Jones, M.D., Steven Shak, M.D., Hank Fuchs, M.D., Virginia Paton, Pharm.D., Alex Bajamonde, Ph.D., Thomas Fleming, Ph.D., Wolfgang Eiermann, M.D., Janet Wolter, M.D., Mark Pegram, M.D., Jose Baselga, M.D., and Larry Norton, M.D.;N Engl J Med 2001; 344:783-792.

32. Neoadjuvant treatment with trastuzumab in HER2-positive breast cancer: results from the geparQuattro study; M Untch, M Rezai, S Loibl, PA Fasching .

33. BRCA1 gene breast cancer; Eliot M.Rosen, Saijun Fan, Richard G. Pestell and Itzhak D. Goldberg ;Journal of Cellular Physiology Vol 196 Issue 1

34. Twenty-Year Follow-up of a Randomized Trial Comparing Total Mastectomy, Lumpectomy, and Lumpectomy plus Irradiation for the Treatment of Invasive Breast Cancer;Bernard Fisher, M.D., Stewart Anderson, Ph.D., John Bryant, Ph.D., Richard G. Margolese, M.D., Melvin Deutsch, M.D., Edwin R. Fisher, M.D., Jong-HyeonJeong, Ph.D., and Norman Wolmark, M.D. N Engl J Med 2002; 347:1233-1241

35. Psychological and social adjustment to mastectomy. A two-year follow-up study Tina Morris BA, H. Steven Greer MD, FRCPsych and Patricia White BTech.

36. Mastery of surgery by fischer 6th edition.

37. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes Prof Umberto Veronesi, MD, GiovanniPaganelli, MD, VivianaGalimberti, MD, Giuseppe Viale, FRCPath, Stefano Zurrida, MD, Marilia Bedoni, MD.

38. The First Organometallic Selective Estrogen Receptor Modulators (SERMs) and Their Relevance to Breast Cancer; The First Organometallic Selective Estrogen Receptor Modulators (SERMs) and Their Relevance to Breast Cancer; Jaouen, Gerard; Top, Siden; Vessieres, Anne; Leclercq, G.; McGlinchey, Michael J.

39. Trastuzumab and Breast Cancer;NEngl J Med 2001; 345:995-998.

40. Chemotherapy of breast cancer Lawrence S. Perlow, James F. Holland.

41. Long-term follow-up of patients with complete remission following combination chemotherapy for metastatic breast cancer.P A Greenberg, G N Hortobagyi, T L Smith, L D Ziegler, D K Frye and A U Buzdar.

42. Estrogen receptor and progesterone receptor status in breast cancer in relation to age, histological grade, size of lesion and lymph node involvement.Sofi GN[1], Sofi JN, Nadeem R, Shiekh RY, Khan FA, Sofi AA, Bhat HA, Bhat RA.

43. Immunohistochemical profile of breast cancer patients at a tertiary care hospital in South India;Ambroise M[1], Ghosh M, Mallikarjuna VS, Kurian A.

44. Comparison of ER, PR and HER-2/neu (C-erb B 2) reactivity pattern with histologic grade, tumor size and lymph node status in breast cancer.Azizun-Nisa, Bhurgri Y, Raza F, Kayani N.

45. Alterations of estrogen receptors, progesterone receptors and c-erbB2 oncogene protein expression in ductal carcinomas of the breast.Hussein MR, Abd-Elwahed SR, Abdulwahed AR.

46. Breast Cancer Survival Defined by the ER/PR/HER2 Subtypes and a Surrogate Classification according to Tumor Grade and ImmunohistochemicalBiomarkers;Carol A. Parise and Vincent Caggiano.

47. The role of human epidermal growth factor receptor 2 in the survival of women with estrogen and progesterone receptor-negative, invasive breast cancer: The California Cancer Registry, 1999–2004 Monica Brown PhD, Katrina R. Bauer MS, CTR Carol A. Parise PhD.

48. Hormone-related Factors and Risk of Breast Cancer in Relation to EstrogenReceptor and Progesterone Receptor Status;Wen-Yi Huang, Beth Newman, Robert C. Millikan, Michael J. Schell,Barbara S. Hulka and Patricia G.

49. Estrogen Receptor – Positive, Progesterone Receptor – Negative Breast Cancer: Association With Growth Factor Receptor Expression and Tamoxifen Resistance ; GraziaArpino , Heidi Weiss , Adrian V. Lee , Rachel Schiff , Sabino De Placido , C. Kent Osborne , Richard M. Elledge.

50. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogeneDJ Slamon, GM Clark, SG Wong, WJ Levin, A Ullrich, WL McGuire.

51. Receptor conversion in distant breast cancer metastases Laurien DC Hoefnagel, Marc J van de Vijver, Henk-Jan van Slooten, Pieter Wesseling, JelleWesseling.

APPENDIX

PROFORMA

QUESTIONNAIRE

NAME:

AGE:

SEX:

IP NO:

OP NO:

DOA:

HISTORY (ONSET, DURATION AND SITE):

PAST / PERSONAL / MARITAL AND MENSTUAL HISTORY IF RELEVANT:

PHYSICAL EXAMINATION (SIZE, NODAL STATUS AND METASTATIC DISEASE FINDINGS):

MAMMOGRAM/USG BREAST:

FNAC/BIOPSY:

CXR,LFT,USG ABDOMEN & PELVIS:

MRI/PET (if any):

NEOADJUVANT CHEMOTHERAPY (if any)

SURGERY PLANNED:

HISTOPATHOLOGY REPORTS:

ER/PR/HRSTATUS:

MASTER CHART

STAGE	ER	PR	HR	TYPE OF CARCINOMA	AGE
EBC	negative	negative	positive	Infiltrating ductal carcinoma	50
EBC	negative	negative	positive	Infiltrating ductal carcinoma	49
EBC	positive	negative	positive	Invasive ductal carcinoma	42
EBC	positive	negative	negative	Invasive ductal carcinoma	60
EBC	negative	negative	positive	Infiltrating ductal carcinoma	55
EBC	positive	positive	negative	Infiltrating ductal carcinoma	55
EBC	negative	negative	positive	Infiltrating ductal carcinoma	43
EBC	negative	negative	positive	Infiltrating ductal carcinoma	45
EBC	positive	positive	negative	mixed invasive and mucinous cacinoma	63
EBC	positive	positive	positive	Infiltrating ductal carcinoma	54
LABC	positive	negative	positive	Infiltrating ductal carcinoma	83
LABC	positive	positive	positive	Infiltrating ductal carcinoma	40
LABC	positive	negative	negative	Apocrine carcinoma	38
LABC	negative	negative	positive	Infiltrating ductal carcinoma	49
LABC	positive	negative	positive	Invasive ductal carcinoma	66
LABC	positive	positive	positive	Invasive ductal carcinoma	67
LABC	positive	negative	negative	Invasive ductal carcinoma	54
LABC	positive	positive	positive	Infiltrating ductal carcinoma	37
LABC	negative	negative	positive	Invasive ductal carcinoma	28
LABC	negative	negative	positive	Invasive lobular carcinoma	65
METASTATIC	positive	negative	positive	Invasive papillary carcinoma	53
METASTATIC	negative	negative	negative	Poorly differentiated squamous cell carcinoma	76
METASTATIC	positive	positive	negative	Infiltrating ductal carcinoma	62
METASTATIC	negative	negative	positive	Infiltrating ductal carcinoma	55
METASTATIC	negative	negative	negative	Infiltrating ductal carcinoma	53
METASTATIC	negative	negative	positive	Infiltrating ductal carcinoma	48
METASTATIC	negative	negative	positive	Invasive apocrine carcinoma	75
METASTATIC	negative	negative	negative	Infiltrating ductal carcinoma	37
METASTATIC	positive	negative	positive	Infiltrating ductal carcinoma	62
METASTATIC	positive	positive	positive	Infiltrating ductal carcinoma	45